THIN-FILM PERIPHERAL NERVE ELECTRODE:

Fabrication of Nerve Cuffs and Evaluation of Selective Stimulation and Somatosensory Neuroprostheses in a Raccoon Model

Final Report

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1.0 OVERVIEW

The objective of the program was the development of thin film nerve cuff electrodes and the demonstration of the efficacy of the electrodes for grasp in an *in vivo* study using a raccoon model. Materials development and fabrication of the cuffs was conducted at EIC Laboratories Inc, while animal studies were conducted at the Rehabilitation Research and Development Center at the Hines VA Hospital (Hines, IL). The cuff electrodes were fabricated by vacuum depositing metal films on thin sheets of FEP Teflon and photolithographically patterning the leads and charge injection sites. The patterned substrate was then thermally sealed with a second polymer layer to electronically isolate the leads from the physiological environment. Once all planar fabrication processes, i.e., photolithography, vacuum deposition, and etching were completed, the electrode was cut out of the substrate and the desired cuff and lead geometries created by thermoforming.

The raccoon was evaluated as an animal model for upper extremity neural prosthesis through anatomical study of the raccoon forearm and stimulation of grasp function using percutaneous intramuscular electrodes and cuff electrodes on the median nerve. Cuff electrodes, implanted on the median nerve were evaluated in acute and chronic studies for selective activation of pronation and grasp. The raccoon was also evaluated as a model for somatosensory neural prostheses.

The specific findings of the program are as follows:

- FEP Teflon can be coated with adherent metal films of Ti, Au, and Ir using DC sputtering. Adhesion is achieved by depositing an initial Ti layer with RF substrate biasing. The substrate bias promotes bombardment of the Ti film by energetic plasma species which, we hypothesize, promotes adhesion by mechanically mixing the Teflon-Ti interface. Using this approach multi-layer metallization of Ti/Ir/Au/Ti films with a total thickness of ~250 nm was deposited on the FEP Teflon.

- Metallized and photolithographically patterned FEP Teflon can be thermoformed into a variety of shapes by autoclaving in the presence of H_2O vapor. The metallization remains adherent during the thermoforming process.

- A separate and graded response to stimulation was observed for forearm pronation and wrist flexion in the raccoon using visual observations of movement, EMG, and torque measurements. The raccoon appears to be a suitable, although challenging, animal model for the development of FES prostheses for restoration of grasp.

- The raccoon model presented two unanticipated difficulties. The median nerve in the upper arm is bifurcated with fascicles within each branch innervating different muscle groups in the hand and forearm. It was therefore not possible to demonstrate that individual fascicles within the same nerve could be selectively activated with the electrodes. A less serious problem was the limited dexterity in the raccoon hand. Selective activation of individual digits were not possible because of the tendon structure in the hand which precludes individual digit motion. The model does, however, provide for separation of pronation and wrist flexion.

- In acute studies of multi-electrode cuffs, with both branches of the median nerve running through the cuff, it was possible to selectively activate grasp and pronation and to obtain gradation in the response.

- Chronic studies revealed problems with the electrode design. All electrodes, even those sutured closed around the nerve, were found, at explanation, to have come off the nerve or to have opened sufficiently to allow significant formation of connective tissue between the cuff and nerve. There is considerable motion between the nerve and surrounding muscle in the upper arm and a more robust scheme for preventing the implanted electrodes from opening would have been desirable.

Work supported by this program resulted in the publication or submission for publication of the following articles:

Scarpine, V., Griffith, P., Walter, J. S., McLane, J. A., *An animal model for upper limb neural prosthesis*. J. Spinal Cord Medicine **18(4)**:264, 1995.

Walter, J. S., McLane, J., Cai, W., Khan, T., Cogan, S. F., *Evaluation of Thin-Film Peripheral Nerve Cuff Electrode*. J. Spinal Cord Medicine,: **18,** 27-31 (1994).

Walter, J. S., Griffith, P. Scarpine, V., Bidnar, M., Dauzvardis, M., Turner, M., McLane, J., Sweeney, J., Robinson, C. J., *The Raccoon as an Animal Model for Upper Limb Neural Prosthetics*, J. Spinal Cord Medicine, **18,** 27-31 (1994).

Walter, J. S., Griffith, P., Sweeney J., Scarpine V., Bidnar, M., McLane, J., Robinson, C. J., *Multielectrode Nerve Cuff Stimulation of the Median Nerve Produces Selective Movements in a Raccoon Animal Model,* submitted for publication.

2.0 ELECTRODE FABRICATION AND *IN VITRO* TESTING

2.1 Electrode Design

During the course of the Phase II effort, three electrode designs were fabricated and evaluated by implantation on the median nerve in the raccoon upper arm. The layout of these electrodes, in planar geometry prior to thermoforming is shown in Figs. 2.1-3. Designs with four circumneural electrodes or with longitudinal tripoles and a steering electrode were fabricated.

2.2 Electrode Fabrication

The nerve cuffs were fabricated from 0.002 inch thick FEP Teflon. Prior to metallization the Teflon substrate was cleaned following the procedure described in Table 2.1.

Table 2.1 Procedure for cleaning FEP Teflon prior to metallization.

Step 1	Immerse 5 minutes in H_2SO_4:H_2O_2; 2:1; H_2O_2 is 30% by weight
Step 2	Rinse in deionized H_2O
Step 3	Dry with cleanroom grade towels (polymer based)

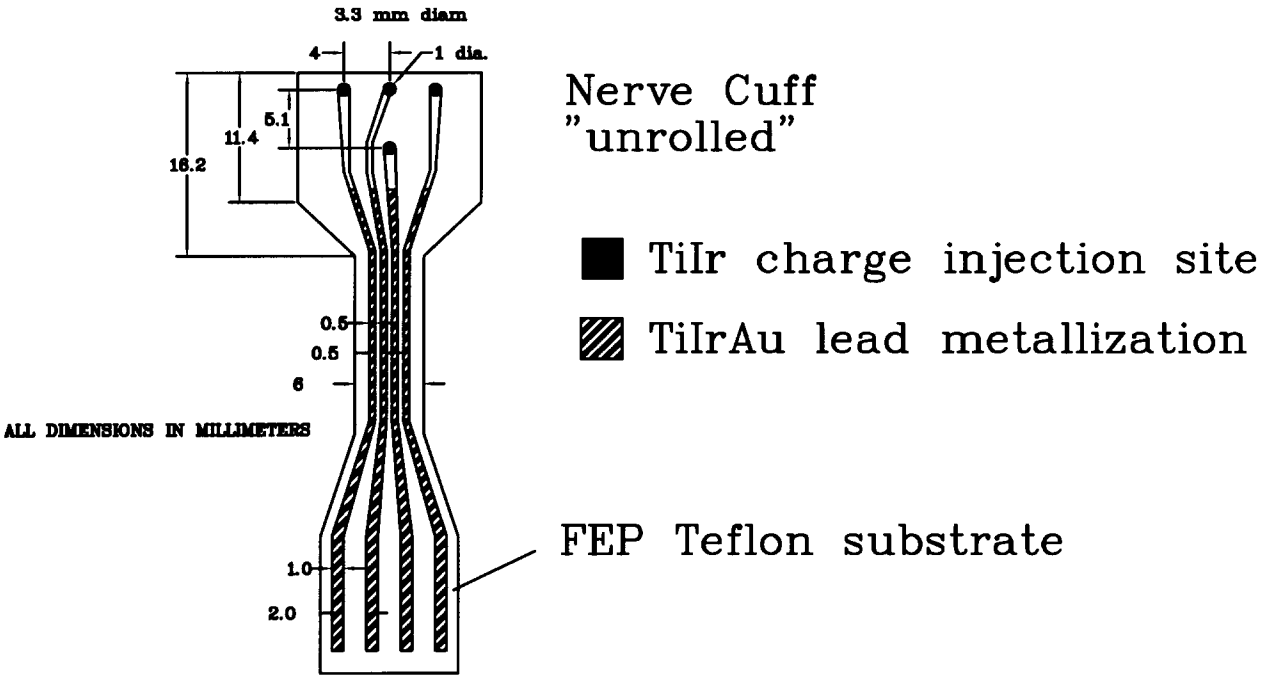

Figure 2.1 Design of an electrode used in acute studies of selective activation on the raccoon median nerve branches. There are four charge injection sites: a longitudinal tripole and a transverse steering electrode.

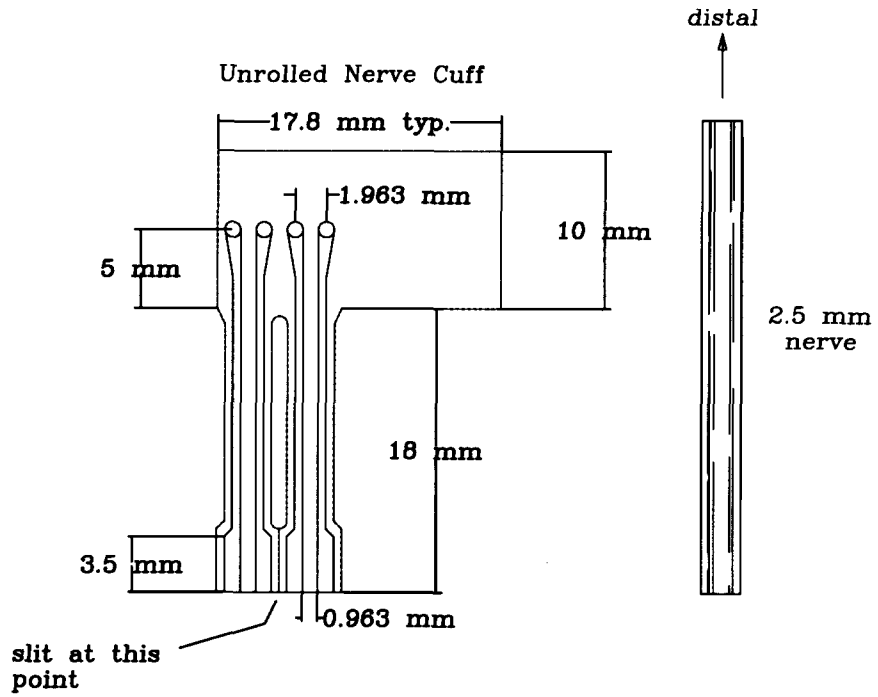

Figure 2.2 Design of an electrode used in acute and chronic studies of selective activation on the raccoon median nerve branches. There are four charge injection sites arranged circumferentially with one electrode in each quadrant.

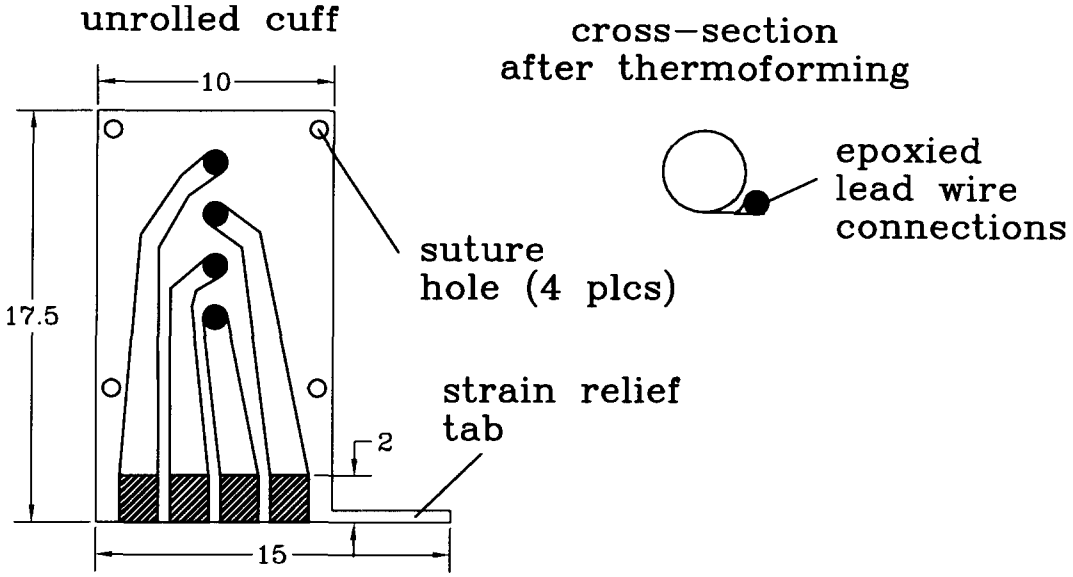

Figure 2.3 Design of the electrode used in chronic studies of selective activation on the raccoon median nerve branches. There are four charge injection sites arranged circumferentially with one electrode in each quadrant.

The cleaned FEP Teflon is taped onto ITO-coated glass (ITO, tin-doped indium oxide). The ITO is electrical conductive with a sheet resistance of 15 Ω/sq. and acts as an electrode for substrate biasing during deposition of the initial Ti adhesion layer. The substrates are plasma cleaned in oxygen for 15 minutes using a low power barrel reactor. The plasma treatment enhances the adhesion and flow characteristics during spinning of the photoresist used to define the pattern of the metallization. The photolithography procedure is outlined in Table 2.2.

Table 2.2 Procedure for photolithographic patterning of FEP Teflon prior to metallizing.

Photoresist	Shipley 1818 microposit (positive resist)
Spin conditions	3500 rpm for 25 seconds
Soft Bake	30 minutes at 100°C
UV exposure	4 seconds (Oriel illuminator)
Developer	Shipley CD-30 developer for 45 seconds
Rinse	Flush with deionized H_2O

A pin connector is attached to the ITO coating on each glass substrate holder using silver epoxy with a second, nonconductive epoxy overlayer to shield the Ag from the plasma. Insulated wires are then attached to each substrate for bias sputtering. Prior to metallization, the substrates are subjected to an *in vacuo* anneal and plasma cleaning following the conditions in Table 2.3.

Table 2.3 *In vacuo* precleaning procedure.

Substrate mounting	Substrates mounted 6.5 cm from sputter targets
Initial pumpdown	Chamber pumped to low 10^{-6} torr range
Thermal anneal	During pumpdown substrates heated 10 min. at 90-120°C and held for 15 minutes at 125°C
Plasma clean	O_2 plasma clean each substrate at 10 watts RF, 10 sccm O_2, 10 millitorr pressure, for 2 minutes
Repump	Chamber pumped to low 10^{-6} torr range

A thin layer of Ti is applied with a RF substrate bias to aid in adhesion at the metal-Teflon interface. To promote uniform coating and minimize damage to the substrates from energetic bombardment during bias sputtering, this initial deposition is done with the substrate moving through a short arc beneath the sputtering gun. The remaining metallization is deposited without a substrate bias. Interfaces between subsequent metal layers are compositionally graded to improve adhesion. Deposition conditions for the metallization are detailed in Tables 2.4-2.6.

Table 2.4 Sputter procedure for Ti adhesion layer on FEP Teflon.

Gas pressure	10 millitorr Ar
Flow rate	10 sccm
DC power	150 mA current, 300-310 V target bias
Substrate bias	5 W RF (13.56 MHz)
Presputter time	10 minutes
Deposition time	6 minutes

Following deposition of the Ti adhesion layer, the chamber is vented to atmospheric pressure with dry nitrogen and the electrical leads for substrate biasing disconnected. A Ti/Ir bilayer is then deposited over the entire area of the cuff using the conditions described in Table 2.5.

Table 2.5 Sputter procedure for Ti/Ir bilayer on the Ti adhesion layer.

Base pressure	Low 10^{-6} torr
Gas pressure	10 millitorr Ar
Flow rate	10 sccm
DC power	150 mA total current (Ti and Ir)
Presputter time	10 minutes
Deposition time	6 minutes
Rotation	105 rpm

The Ti-Ir interface, is graded by sputtering simultaneously from two targets and systematically increasing the sputtering current at one target and decreasing it at the other, while maintaining the total current constant. The planet with the Teflon substrates is spun underneath the two targets during deposition at a speed of 105 rpm to ensure uniform coating. Following deposition of the Ir, the chamber is vented to atmosphere and the area of metallization that will be used as a charge injection sites is masked with a thin glass microscope slide. A Ti/Au bilayer is deposited over the remaining metallization to reduce the resistance of the lead lines. The deposition conditions for the Ti/Au are provided in Table 2.6. The overall sequence of metals in the coating is the following:

Charge injection site: FEP-Teflon/Ti(w/bias)/TiIr graded interface/Ir;

Lead metallization: FEP-Teflon/Ti(w/bias)/TiIr graded interface/Ti/TiAu graded interface/Au.

Table 2.6 Sputter procedure for Ti/Au bilayer on Ir.

Base pressure	Low 10^{-6} torr
Gas pressure	10 millitorr Ar
Flow rate	10 sccm
DC power	150 mA total current (Ti and Au)
Presputter time	10 minutes
Deposition time	6 minutes
Rotation	105 rpm

Photoresist and unwanted metallization is removed by liftoff using acetone. Light mechanical abrasion or ultrasonic agitation is occasionally necessary to remove all the residual resist.

A silicone polymer, MED-6605 with primer SP-135 from NuSil Silicone Technology (Carpartina, CA), was used as the inner insulation on the cuff electrodes. The polymer was spun coated over the metallization and cured at room temperature. The details of the application and curing procedure are provided in Table 2.7.

Table 2.7 Application and curing procedure for MED 6605 on FEP Teflon.

Step 1.	Mask charge injection sites and bonding pads with tape
Step 2.	Apply NuSil SP-135 primer by spin coating at 3000 rpm for 20 s
Step 3.	Cure primer by exposure to moist atmosphere (RH >50%) for 1-2 hr
Step 4.	Apply NuSil MED 6605 by spin coating at 2000 rpm for 20 s
Step 5.	Remove tape masks
Step 6.	Cure at room temperature for 1 week

The cuff is formed in the coated FEP Teflon sheet by annealing in an aluminum mold at 135°C. The operation is performed in an autoclave with H_2O present. The H_2O in the "steam annealing" process is to improve heat transfer and is not thought to modify the properties of the FEP Teflon (e.g. act as a plasticizing agent). The cuffs are autoclaved for 30-40 minutes. After autoclaving, the cuffs and mold are quenched in cold water.

2.3 Electrochemical Characterization

The electrochemical behavior of the Ir electrode sites was evaluated by cyclic voltammetry and charge-injection in a two-buffer saline electrolyte. The electrolyte contained carbonate and phosphate buffers in the same composition found in interstitial fluid (1); 137 mM NaCl, 29 mM $NaHCO_3$, 1.7 mM Na_2HPO_4 and 0.7 mM NaH_2PO_4 purged with a 5% CO_2/6% O_2/89% N_2 gas mixture to a pH of 7.4. Connection to the individual electrodes was made by attaching stainless steel wire to the bonding pads with electrically conductive epoxy. The electrochemical cell contained a saturated calomel reference electrode (SCE) for potential measurements and a Pt mesh counter electrode.

An initial cyclic voltammogram of a charge injection site without activation is shown in Fig. 2.4. The electrode was cycled between limits of -0.6 and 0.8 V vs SCE at a sweep rate of 50 mV/s. The large currents on the cathodic sweep are from reduction of oxygen from the sparge gas. After cycling for 40 minutes between these potential limits, activation of the electrode is observed as shown by the CV in Fig. 2.5. A distinct reduction-oxidation wave develops in the 0.0 V to 0.4 V potential range and the reduction at the negative potential limit is greatly reduced. After 100 minutes of cycling, shown in Fig. 2.6, the feature of the CV become similar to Ir wire electrodes activated in the same electrolyte.

After 18 hr of continuous cycling, the charge capacity at a single electrode site had increased during a 50 mV/s CV to about 5 mC/cm^2. A CV of an electrode site with a capacity of 5.1 mC/cm^2 on the cathodic sweep is shown in Fig. 2.7. The "noisy" current response is due to the gas mixture bubbling into the electrolyte. The charge injection characteristics of the electrode were evaluated using monophasic, capacitively coupled pulses at a repetition rate of 60 pps and pulse width of 100 μs. The anodal and cathodal charge capacity, interpulse potential (IPP), access resistance (R_a), maximum current (I_{max}), and open-circuit potential after 2 minutes without pulsing (V_{oc}) are listed in Table 2.8. The maximum charge capacity is defined as that which produces a potential transient of -0.6 and 0.8 V vs SCE on cathodal and anodal pulsing, respectively.

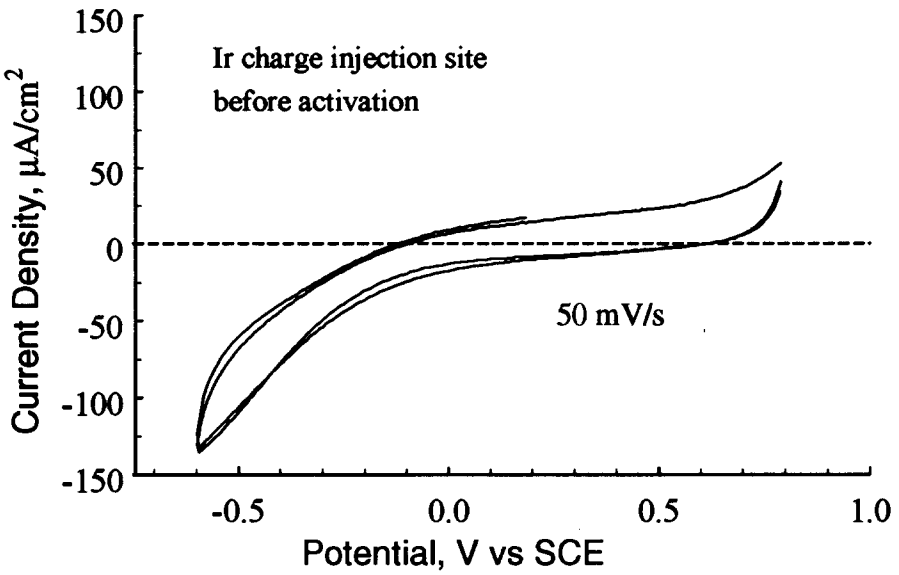

Figure 2.4. Cyclic voltammogram of an Ir charge injection site prior to activation.

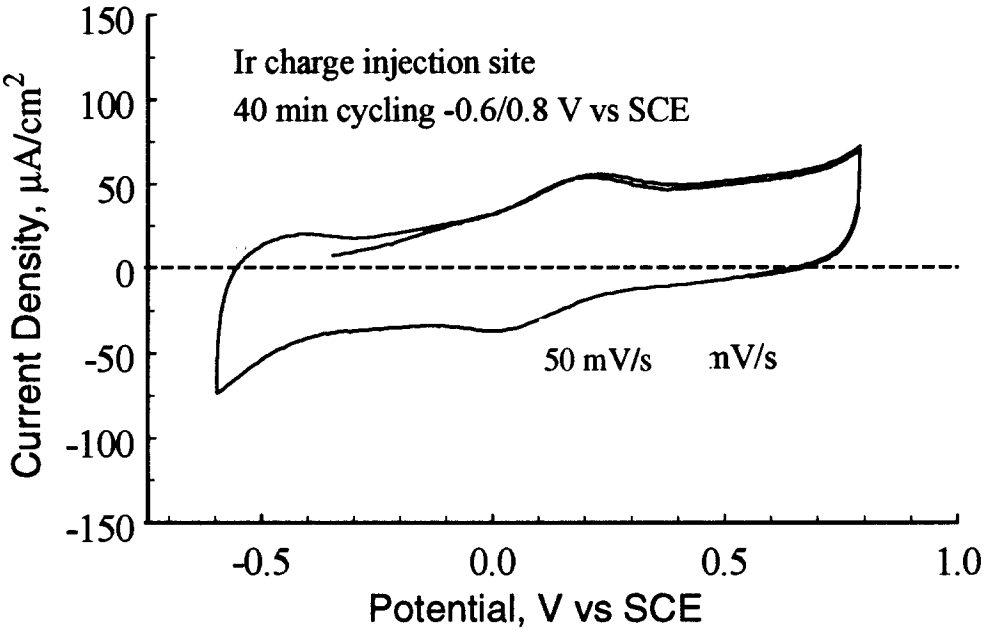

Figure 2.5 Cyclic voltammogram of an Ir charge injection site after activation by 40 minutes of cycling between limits of -0.6 V and 0.8 V vs SCE at 50 mV/s.

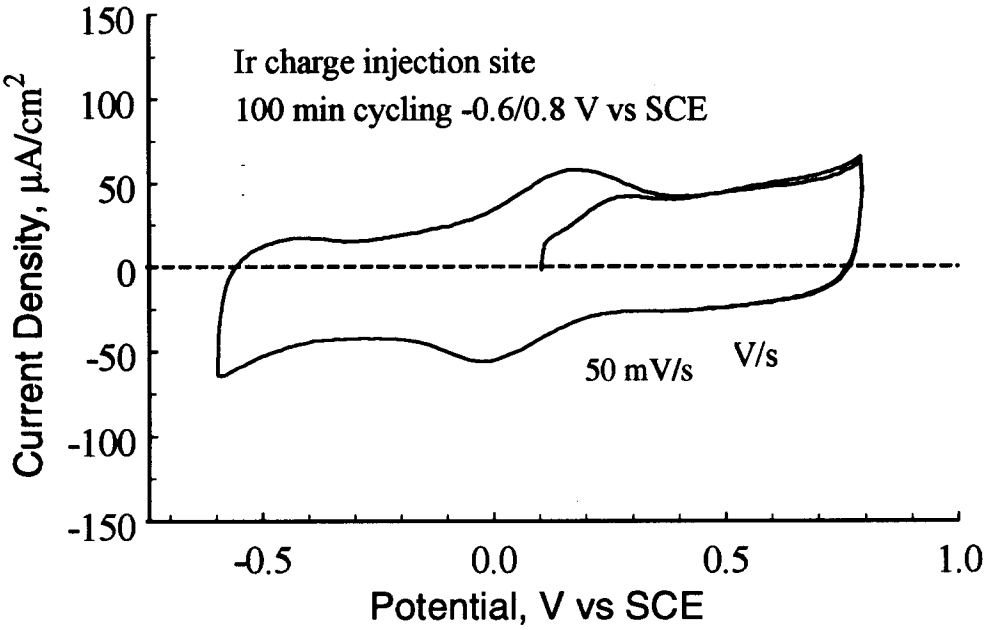

Figure 2.6 Cyclic voltammogram of an Ir charge injection site after activation by 100 minutes of cycling between limits of -0.6 V and 0.8 V vs SCE at 50 mV/s.

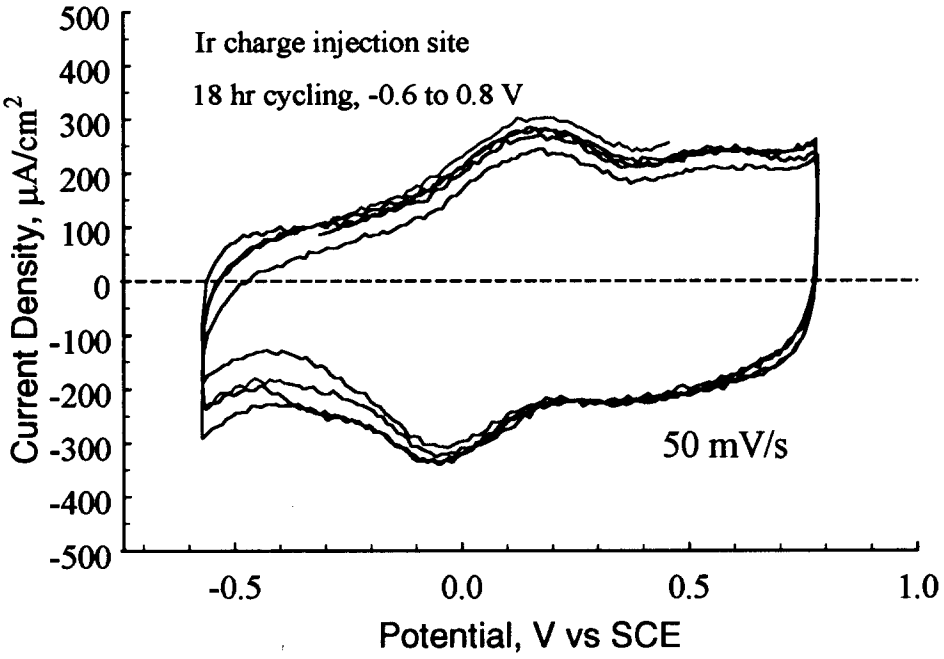

Figure 2.7 Cyclic voltammogram of an Ir charge injection site after 18 hr cycling between limits of -0.6 and 0.8 V vs SCE at 50 mV/s.

Table 2.8 Charge injection characteristics of an Ir electrode site after 18 hours of potential cycling in PBS/CBS between -0.6 V and 0.8 V vs SCE.

Pulse Direction	I_{max} mA	Charge $\mu C/cm^2$	IPP V vs SCE	R_a Ω	V_{oc} V vs SCE
Anodal	7.6	97	0.05	87	0.08
Cathodal	8.6	110	0.04	74	0.05

The electrode was then activated by potential cycling at 50 mV/s between limits of -0.82 V and 0.96 V vs SCE. After 30 minutes of activation, the cathodic charge capacity during cyclic voltammetry (-0.6 to 0.8 V vs SCE limits) doubled to ~10 mC/cm^2. The corresponding charge capacity during pulsing, detailed in Table 2.9, increases to 190 $\mu C/cm^2$. This electrode was then pulsed continuously for 16 hr with 10 mA (130 $\mu C/cm^2$) cathodal pulses. The initial and final charge injection characteristics with the 10 mA pulses are listed in Table 2.10. The maximum cathodic potential excursion (E_{mc}), corrected for the access voltage, decreased from -0.380 V to -0.320 V during the pulsing. Comparison of the CV response after pulsing, shown in Fig. 2.8, shows little change in electrode behavior from that prior to pulsing (Fig. 2.7), except for a modest increase in charge capacity that is presumably due to further activation of the Ir.

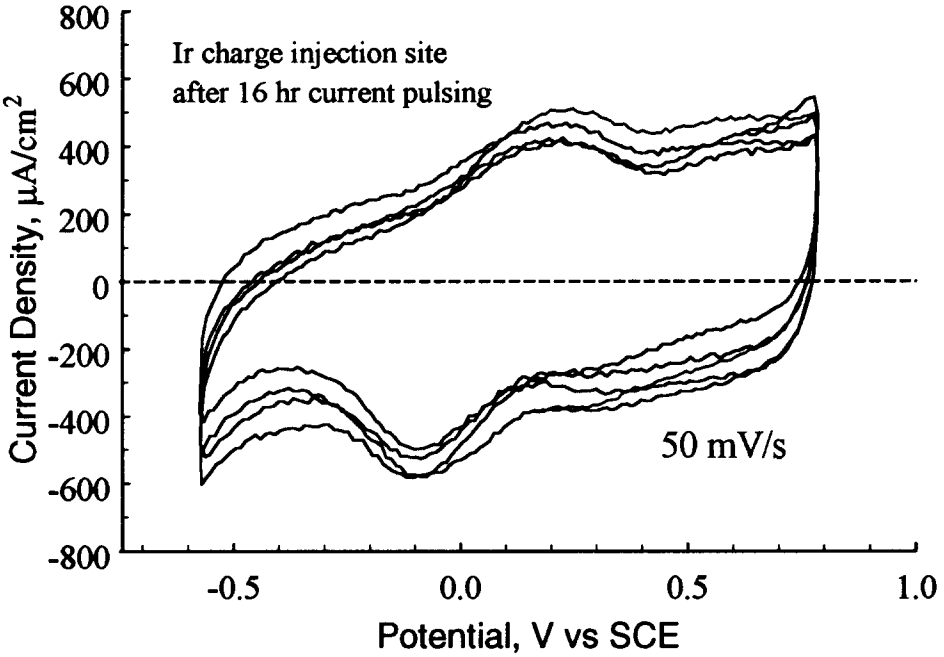

Figure 2.8 Cyclic voltammogram of an Ir charge injection site after 16 hr of cathodal pulsing at 10 mA.

Table 2.9 Charge injection characteristics of an Ir electrode site after 30 minutes of pulse activation in PBS/CBS between -0.82 V and 0.96 V vs SCE.

Pulse Direction	I_{max}, mA	Charge $\mu C/cm^2$	IPP V vs SCE	R_a Ω	V_{oc} V vs SCE
Anodal	15	191	0.07	75	0.08
Cathodal	14.8	189	0.06	70	0.08

Table 2.10 Charge injection characteristics of an activated Ir electrode site after 16 hours of continuous cathodal pulsing at 10 mA.

Time, hr	E_{mc} V vs SCE	IPP V vs SCE	Access ResistanceΩ	V_{oc} V vs SCE
0	0.38	0.08	72	0.08
15.5	0.32	0	68	0.08

The electrochemical characterization of the cuff electrodes was complicated by a difficulty in achieving full electrolyte access to the inner surface of the cuffs during testing. The hydrophobic inner surface allowed air to remain trapped at the electrode sites following immersion in the electrolyte. To avoid this problem, a liner of filter paper was saturated with electrolyte and placed inside the cuff. The filter paper expands slightly and wicks electrolyte to the electrode sites. This procedure significantly reduced the occurrence of high resistance sites which, prior to recognizing the problem of air occlusion, had been attributed to fractured metallization.

3.0 *IN VIVO* STUDIES

3.1 Overview of *In Vivo* Studies

Investigations were conducted in four areas. First, the observed dexterity and control of forelimb function led us to select the raccoon as a model for graded independent muscle function. The raccoon volar forearm anatomy is described, as well as paw movements, torque and EMG responses to direct muscle stimulation using percutaneous electrodes. Second, the acute responses of direct median nerve stimulation through an implanted multielectrode nerve cuff has been investigated. A 12-electrode cuff implanted on the median nerve was also evaluated. Paw movements in response to stimulation as well as direct tendon forces were recorded. Third, cortical responses to median nerve stimulation were investigated. Fourth, chronic implantation of an FEP Teflon nerve cuff has been evaluated.

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3.2 The Raccoon as an Animal Model for Upper Limb Neural Prosthetics

Functional Neuromuscular Stimulation (FNS) is being used to improve the ability of individuals with quadriplegia to perform activities of daily living requiring hand-wrist function. Several methods of upper limb FNS are in clinical trials including surface, percutaneous, and implantable electrode systems (2-6). Improvements in implant durability, ease of implantation, and control

systems are being actively pursued (5,6). The cat and dog have been used as a model in some studies (7,8), but these animals have has limited paw and digit movement. Thus, a better laboratory animal model is needed for these research efforts, in that the animal selected should have an upper extremity anatomy similar to that of the human, selective forearm muscle function, and graded muscle response (9). The large dexterous forearms and animal size led us to evaluate the raccoon as an animal model of FNS. In this paper the raccoon volar forearm anatomy is described. Independent muscle response was evaluated by torque and EMG responses to percutaneous muscle stimulation. The advantages of the raccoon as an animal model for upper limb neural prosthetic research are presented.

3.2.1 Methods

Four cadaver and four fresh specimen forearms were dissected. The median nerves were traced to muscle innervation in five animals where median nerve fascicle mapping was performed. Four raccoons were observed in their cages, and the use of forelimbs in feeding, digging, and grasping objects was noted. For electrical stimulation studies, four raccoons (Hummel Creek Kennels, St. Louis, MO) weighing an average of 4.5 ±0.26 Kg (mean ±S.E.M.) were anesthetized with ketamine hydro-chloride (20 mg/Kg) and xylazine (2 mg /Kg, IM body weight). Anesthesia was maintained with Ibuphronex (IM) and Phenobarbital (IP) in order to prevent ketamine-induced spasms. Percutaneous electrodes were used for stimulation and EMG recording. The electrodes were single strands of 316 stainless steel wire (0.006 inches diameter) insulated with a thin coating of polyamide except for the last 3 mm of exposed wire (Life-Tech Inc., Houston, TX). The electrodes were inserted with a 27 gauge needle. Two stimulating electrodes were inserted in each muscle, separated by approximately 3 mm. Two recording electrodes were inserted in the same muscle through a single 23 gauge needle approximately one cm distal to the recording electrodes.

Four sets of stimulating electrodes were implanted in the volar forearm (with the location determined from dissections and from preliminary stimulation studies). Electrodes were withdrawn and reinserted if stimulation did not produce the expected paw movements. The location of the electrodes was measured lateral and distal to the insertion of the biceps muscle.

The first three electrodes were inserted into superficial muscles to a depth of 3 mm after passing through the skin. These muscles were pronator teres (PT) (1 cm lateral to the biceps insertion), flexor carpi radialis (FCR) (1.5 cm lateral), and a closely spaced pair of muscles consisting of flexor palmaris longus/flexor digitorium superficialis (FPL/FDS) (3.5 cm lateral, 2 cm distal to the biceps insertion). The fourth set of electrodes were implanted in the deep muscle flexor digitorium profundus (FDP) (3.5 cm lateral, 2 cm distal and 2 cm deep). Movements were observed following single stimulation pulses at threshold, half-maximal and maximal-stimulating currents. Several other techniques were used to help distinguish which muscles were being characterized; i.e., the tendons were palpated in the forearm, the palm of the paw was held firm by an investigator so that digit flexion could be observed without wrist movement, and sustained muscle contraction response to repeated stimulus pulse trains was observed.

Two sets of EMG recording electrodes were placed in the forearm muscles. The first set was placed in the muscle that showed the strongest pronation response to the stimulating electrodes, either PT or FCR. The second set was placed in the muscle that showed the strongest digit flexion to the stimulating electrodes, either the FPL/FDS or FDP. The EMG signals from the recording electrodes were amplified with preamplifiers (Gould Universal Amplifier) and observed on an oscilloscope (Gould Oscilloscope with printer, Cleveland, OH). Recruitment to increasing stimulating currents of the EMG signal was observed and printed on the plotting oscilloscope. Further processing of the EMG signal allowed for simultaneous printing of the full-wave rectified and integrated EMG signal. The EMG signal was passed through a signal artifact suppression unit (Fredrick Heir Artifact Suppression Unit, Brunswick, ME) and through a signal rectifier and integrator unit (Gould Integrator Unit) before being displayed on a stripchart recorder (Astro-med, West Warwick, RI). Stripchart recordings of the integrated EMG signal were obtained with stimulating currents ranging from threshold to maximum. Stimulating pulse trains for the stripchart recordings were 20 pulses per second (pps), 100 μs pulse duration applied for 1 sec. Two minutes of recovery time were allowed between each stimulation train.

Isometric torques of the paw or digits to one-second stimulation trains were also recorded. For recording wrist and digit isometric torque responses, a hinged two-plate system was used. The first plate was fixed to a rod that was anchored to the table. The forearm was then anchored to

this plate with a strap. The hinge was at the level of the metacarpal-phalangeal joint for recording digit flexion and at the wrist for recording flexion at this joint. The second plate was secured with tape to the distal digits or paw and connected to a force transducer (Grass Force transducer, Quincy, MA) with braided umbilical string having minimal stretch. The string was attached to a hole in the second plate, 4.5 cm from the hinge. For pronation recordings, a plaster cast extending halfway around the forearm was taped in place. Torque measures were recorded from a 4.5 cm rod extending out from the plaster cast and attached to the force transducer with the umbilical string. The forearm was allowed to rotate freely but was blocked from lateral and vertical movement with bars. Oil was placed on the cast to reduce any friction between the bars and cast. The position of the anesthetized animal reclining on its test side against its upper arm provided further stabilization of the test arm. Initial conditions were optimized for each of the isometric torque measures. The joint position was fully extended with slight tension for wrist and digit flexion measurements. For pronation measurements, the initial condition was supinated with slight tension. Force transducers were calibrated with free hanging weights and the forces were displayed on the stripchart recorder. Wrist flexion, digit flexion, and pronation torques were recorded across the same range of stimulating currents used for the EMG stripchart recordings. Torque measures were obtained as the moment of force (distance times force) where the distance was 4.5 cm for all of the torque recordings. For simplification, only forces in milliNewtons (mN) are presented (torques in units of mN-cm can be determined by multiplying the force by 4.5 cm)

3.2.3 Results of Anatomical and Functional Characterization of the Raccoon Model

Volar Forearm Anatomy. There were several distinguishing features in the raccoon forearm. The primary origin of FPL is on the proximal ulna with a secondary origin on the radius. Unlike the human, the raccoon FDS and FDP tendons are not differentiated into individual tendons at the wrist. Rather, the FDS and FDP digit tendons are only distinct proximal and distal to the wrist. The lumbrical tendons also appear as a sheet on the FDP tendons.

The palm in such adult animals measures approximately 2.5 by 2.5 cm and 1 cm thick. The digits extend out an additional 3 cm and are easily distinguishable. The palm is well developed with

thenar, hypothenar, and interosseous muscles. However, the thumb is the smallest of the digits. The paw intrinsic muscles are primarily innervated by the ulnar nerve.

The raccoon median nerve does not combine from the medial and lateral cords in the brachial plexus as it does in humans. The cords remain divided in the upper arm with a smaller superficial more ulnar branch, and a larger deep branch that are found between the musculocutaneous and ulnar nerves. All four nerves are ulnar to the biceps muscle. The two median nerve cords combine in the mid-forearm after providing innervation to volar forearm muscles. The deep branch of the median nerve passes through a supracondylar foramen such as the one seen in cats. After motor innervation is given the cords combine to provide sensation to the radial three digits of the paw. The motor branches were tagged and the fascicles were mapped. There was some duplication of function and cross innervation seen in some specimens such as a median nerve branch in one animal to the FCU. For the five animals that had nerve mapping, the smaller, more-ulnar, and more superficial, median-nerve cord contained PT, FCR, and coracobrachialis motor fibers and sensory fibers to the elbow and paw. The deeper radial median nerve contained FDS, FDP, FPL, FCR and thenar motor fibers, and the majority of sensory fibers to the paw. In all raccoons, FCU and a portion of the FDP received ulnar innervation. Once the motor fibers were given off, the two branches of the median nerve combined in the proximal forearm to provide sensory and motor fibers to the paw. No anterior interosseus nerve was seen.

Functional Observations. The raccoons were observed as they ranged about in their cages and ate. None of the raccoons demonstrated active opposition of the ulnar digits and thumb to hold food or a bar. The thumb is small and had little functional use. A type of passive opposition occurred, however, with spreading of the digits over a bar while standing on it. When the animals climb and hang from a bar, the paw is used like a claw and hooks over the bar. Clawing actions of the paw are also seen using the strong flexor muscles of the forearm when they dig in the cage bedding for food. The raccoon holds food between their two forepaws much like a squirrel. Thus, little individual digit function was noted. Because the function of the paw of the raccoon was more like a claw than the dexterous fingers of the human, further torque studies were limited to pronation of the forearm, wrist flexion and digit flexion. The digits acted in unison so torque studies were conducted with the digits taped and strapped to a single force plate.

Stimulation of intrinsic paw muscle resulted in selective movement of digits. However, due to their small size, intrinsic paw muscles were not suitable for simultaneous stimulation and EMG recording, and they were not further studied. Volar forearm muscles were implanted with stimulating electrodes. Four sets of muscles or muscle groups were stimulated as described in section 3.2.1. A wide range of currents were required to induce a minimum or threshold movement of the forearm or paw. These currents ranged from 0.13 to 2 mA (Table 3.1). Raccoon #3 had particularly low threshold currents. The following responses were typically seen at threshold current; 1) PT induced pronation and some wrist flexion; 2) FCR induced wrist flexion and some pronation; 3) FDS stimulation induced primarily flexion of all of the digits with some wrist flexion; 4) FDP induced digit flexion responses indistinguishable from the FDS group. Because there was some over lapping of movements with the PT/FCR and the FPL/FDS/FDP groups they were evaluated together in Table 3.1. There was a range of currents for the muscles. Stimulating currents to induce maximal movements ranged from 0.28 to 14 mA. These maximal movements were associated with decreased selectivity of motions. Increased digit flexion and some elbow flexion was invariably seen indicating recruitment of some of the major flexor muscles of the forearm.

Table 3.1 Stimulating currents that resulted in threshold and maximal forearm and paw movements.

Raccoon	Muscle	Threshold Response mA	Maximal Response mA
#1	PT/FCR[a]	1.0	8.0
	FDP/FDS/FPL	2.0	6.0-8.0
#2	PT/FCR	1.0	6.0-14.0
	FDP/FDS/FPL	0.8	4.0-8.0
#3	PT/FCR	0.16-0.2	0.28-0.9
	FDP/FDS/FPL	0.13-0.25	0.34-0.9
#4	PT/FCR	0.38-1.5	4.0-10.0
	FDP/FDS/FPL	0.5-1.2	3.4-7.0

[a]Because there was some over lapping of forearm and paw movements, the muscles were grouped together as shown.

EMG and Torque Responses to Stimulation. The two sets of implanted stimulating electrodes that produced the greatest difference in forearm and paw movement were further evaluated with EMG and torque recordings. In most of the studies this consisted of the PT set of electrodes for pronation and either the FPL/FDS or the FDP sets of electrodes for digit flexion. The EMG response usually consisted of a biphasic M wave of short latency (Fig. 3.1A). Multiphasic M waves were also recorded with three to five peaks. The M waves were further electronically processed to display the EMG evoked response on a stripchart recorder. Artifact suppression and rectification of the M wave is shown in Fig. 3.1B, and a typical stripchart recording of the resulting integrated EMG response is shown in Fig. 3.1C.

Isometric force (torque) responses were reproducibly recorded for finger flexion, wrist flexion and forearm pronation. Figure 3.2 shows stripchart records of typical integrated EMG and digit forces to one second of stimulation of the FDS/FPL muscle group. Both EMG and force records show abrupt onsets that are maintained throughout the stimulation period. Both measures increase in response to increased stimulating current.

Selective stimulation of muscles with the percutaneous electrodes was seen with both EMG and isometric force recordings. Stimulation of PT primarily induced an EMG response in PT not FDP. Similar selectivity was observed with FDP stimulation. Averaged responses for the four animals also showed selectivity (Table 3.2). EMG responses from the stimulated muscles were 4 to 10 times larger than the EMG response in the nonstimulated muscle.

Selective stimulation was also seen with isometric force recordings. Pronator teres stimulation resulted in high PT force with little wrist or digit force. Similarly, at the threshold and higher currents, FDP/FDS/FPL stimulation resulted in high wrist and digit flexion forces with little pronation. Table 3.3 summarizes these selective responses based on force records. Again, highly selective stimulation is shown. For example, wrist flexion force with FDP or FPL/FDS stimulation was 6 to 12 times larger than with PT stimulation.

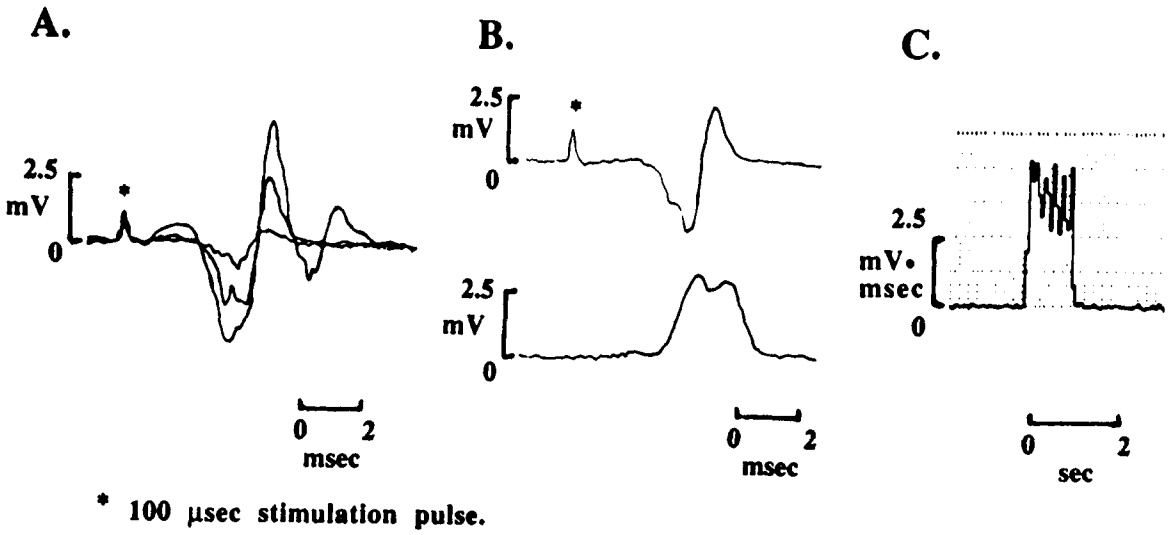

Figure 3.1 Percutaneous stimulation and recording of EMG activity from FCR muscle A. Biphasic M wave including stimulation artifact for threshold, 1/2-maximal, and maximal stimulating current. B. Top waveform is biphasic M wave including stimulation artifact and bottom waveform is rectified with artifact suppression. C. Stripchart recording of integrated M wave as shown in lower record of B from 100 µsec pulses at 20 pulses per second for one second.

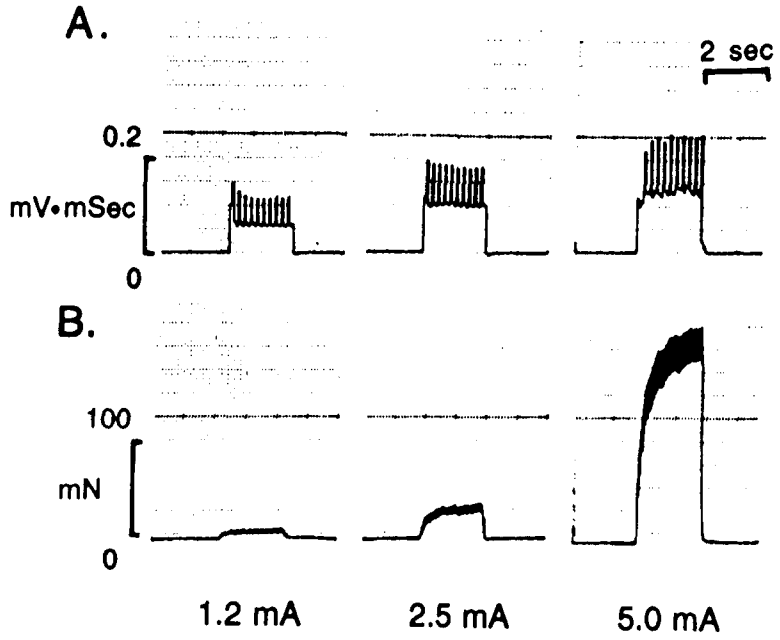

Figure 3.2 Responses to stimulated FPL/FDS at 1,2 mA, 2.5 mA, and 6 mA. A. Integrated EMG of FPL/FDS muscle. B. Digit force (torque) to stimulation. Both EMG and force records show increased recruitment of muscle contraction with increased current strength. Spikes in the EMG record were disregarded as they reflect two stimulus pulses in one integrated interval.

Table 3.2 Selective activation of stimulated volar forearm muscles based on EMG response.

Stimulation of PT/FCR

Current		Activation of PT/FCR	Activation of FDS/FDP/FPL
(mA)	N	EMG (%)[a,b]	EMG (%)
1.9±1.9	4	19.8±1.0	0.0±0.0*
3.5±3.5	4	52±4.2	10.8±9.0*
6.6±6.0	4	100±0	9.8±13.0

Stimulation of FDS/FDP/PL

Current		Activation of FDS/FDP/PL	Activation of PT/FCR
(mA)	N	EMG (%)	EMG (%)
1.5±1.0	4	24.5±5.8	6.4±9.4
2.6±1.7	4	52.8±17.3	9.8±13.0*
7.3±4.8	4	100±0	32.0±38.1*

[a]Although two muscles are indicated, only one muscle was used in an individual raccoon, the muscle giving the greatest separation of EMG responses (see methods).

[b]Percentages were obtained as the ratio of the EMG response in a muscle divided by the maximal EMG response when the same muscle was receiving a maximal stimulation.

*Significantly different from results presented in the same row, $p \leq 0.05$, Student t paired test.

Table 3.3 Selective activation of stimulated volar forearm muscles based on isometric force.

Stimulation of PT/FCR, Pronation Response

Current		Stimulation of PT/FCR	Stimulation of FDS/FDP/FPL
Strength[a]	N	Total Pronation Force, %[b]	Total Pronation Force, %
Low	2	21-32	0-7
Medium	2	30-50	0-5
High	2	86-90	11-14

Stimulation of FDS/FDP, Wrist Force Response

Current		Stimulation of FDS/FDP/FPL	Stimulation of PT/FCR
Strength	N	Total Wrist Force, %	Total Wrist Force, %
Low	4	18±4	3±6*
Medium	4	47±9	4±8*
High	4	90±17	11±17*

Stimulation of FDS/FDP, Digit Force Response

Current		Stimulation of FDS/FDP/FPL	Stimulation of PT/FCR
Strength	N	Total Digit Force, %	Total Digit Force, %
Low	4	20±7	0±0*
Medium	4	52±8	0±1*
High	4	98±4	2±4*

[a]Low current strength was approximately 25% of the current that gave a maximal response, medium was 50% and high was 100%.

[b]Percentage based on the ratio of force/total force where total force is the sum of the maximal forces (highest current) for the two muscles stimulated.

*Significantly different from the other response in the same row of this table, $p^{2}0.05$, Student t test with paired data.

3.2.3 Discussion of Anatomical and Functional Characterization of the Raccoon Model

The raccoon's volar forearm anatomy and physiology indicate that this species may be a better model for testing neuroprosthetics than the cat or dog models. The large digits of the raccoon with their clawing function appear to be more similar to humans than the cat or dog. The muscles of the raccoon forearm are larger than cats or dogs which allowed for the insertion of stimulating electrodes into individual muscles. The raccoon had some similarities to the cat (9,10). The raccoon FPL like the cat is relatively larger than the human, but the FPL in both species connects to palmar fascia. The human FPL origin is primarily the middle third of the radius with a vestigial origin on the proximal ulna. The raccoon FPL has its primary origin on the proximal ulna with a secondary origin on the radius. Other median innervated muscles in the raccoon forearm have similar origins and insertions as in the human. Since only eight raccoons were studied, the occurrence of anatomic anomalies was not strictly determined.

The divided median nerve in the upper arm is a limitation of the raccoon animal model. In contrast to the human with a single median nerve cord in the upper arm, the median nerve cords in the raccoon do not combine in the brachial plexus area but instead combine after forearm innervation is given (11,12). Only the raccoon's deep branch of the median nerve passes through a supracondylar foramen. Felines have the entire median nerve passing through this foramen (9). In most humans the median nerve passes under the ligament of Struthers which in a few cases is calcified. In the raccoon there are some duplication of forearm innervation from the superficial and deep cords of the median nerve. This division of the median nerve and duplication of innervation would be an important limitation to take into consideration for the evaluation of, for example, a median nerve cuff electrode. Divided nerves might be expected to be stimulated more selectively than nondivided nerves because the high resistance perineurium around each cord of the nerve will restrict current flow.

The separate tendons that are bound together at the carpal tunnel and then separate in the palm appear to be an early variant on tendon sheaths. In no raccoon were digit tendons individually gliding. This binding of the tendons resulted in movement of all of the digits together, which probably serves clawing and digging functions. This would also duplicate function in cases of

injury. Fusion of the tendons in the wrist and palm of the paw is also seen in the dog and cat. This is quite distinct from uninjured humans where individual digit movement predominates. In the raccoon, the FDS and FDP tendons adhere to each other as they pass through annular ligaments in the fingers. Separate FDS, FDP insertions were not identifiable even in fresh dissection. Insertions in the digits did not show separate distal interphalangeal flexion from proximal interphalangeal joint flexion. The raccoon has a thick palm and digit pads covering the volar paw which obscure digit fine movements. Finger flexors cross the wrist, and flexion of fingers results in flexion forces across the wrist.

Another major difference from the primate hand is seen with the small thumb of limited function in the raccoon. Passive but not active opposition was observed in this animal. Grasping seen in the raccoon is entirely with the digits acting as a claw. Since grasping with the thumb is an important neural prosthetic application for humans, this animal model like the cat and dog is limited in this area.

Separate graded responses to stimulation were evaluated using three separate measures: visual observations of movements, EMG and torques (13). These measures indicate that basic paw movements can be evaluated in this animal model. These types of selective stimulation have been shown clinically (2,4) and continue to be developed for neuroprosthetic applications. This animal model may be useful in future development of upper limb neuroprosthetic technologies including cuff electrodes, feedback control, and sensors (4,6,7). For example, we are developing a new thin-film nerve cuff electrode to selectively activate digits (14).

The percutaneous electrodes used here are satisfactory for selective stimulation and recording from skeletal muscle (15). These electrodes can stimulate and record from the small muscles of the volar forearm, and these electrodes are commercially available for human applications. We are using these electrodes in other animal studies to stimulate respiratory muscles (16), and to stimulate pelvic nerves for micturition (15,17). Clinical use of percutaneous electrodes can provide acute data for neural prosthetic devices (3,4).

Clinical research allows many opportunities to conduct studies in patient populations. Studies could be conducted in perfused amputated limbs, investigational stimulation can also be done

during routing surgery procedures. However, the time available under these surgical conditions is usually too limited for the extensive studies needed in neuroprosthetic evaluations. Current upper limb implants in individuals with spinal cord injury allow for optimization of stimulating parameters and stimulation protocols (2,4). New neuroprosthetic technology is needed in the areas of improved stimulators and stimulating cables, and electrodes. Closed loop control devices are also needed. Studies of new technology still needs to be evaluated in an animal model. The raccoon appears to be a suitable animal model for development in these areas. Raccoons are relatively disease free and available from reputable vendors that breed animals for research and furriers. The use of squeeze cages allows for easy percutaneous injection so that the animals are readily studied under anesthesia. In comparison to the raccoon, no other nonprimate animal model allows for such extensive evaluation of hand movements.

3.3 Multielectrode Nerve Cuff Stimulation of the Median Nerve

3.3.1 Application of Nerve Cuff Electrodes for Selective Stimulation

Functional Neuromuscular Stimulation (FNS) is a biomedical technology being developed for restoration of limb movement in the neurologically disabled. Selective electrical stimulation for hand movement (i.e. activation of a desired muscle(s) without activation of undesired muscles) can often be achieved using intramuscular or epimysial electrodes (5,18). Lower limb movement restoration systems often use similar electrode technologies (19). Each intramuscular or epimysial electrode activates a- motoneuron fibers as they penetrate into a given muscle (20,21). This approach by definition requires multiple electrode leads to forearm muscles (i.e. at least one electrode and lead per muscle to be stimulated). Not surprisingly, electrode breakage at the level of the elbow has been a reported difficulty due to electrode flexion fatigue. Alternatively, a multielectrode 'cuff' implanted on nerves above the elbow might be able to reproducibly elicit desired volar forearm movements via selective activation of multiple muscles (or muscle groups) by a single implant. This approach relies upon an expectation that fascicles within a nerve trunk be arranged such that each fascicle provides innervation to one muscle (or perhaps one set of muscles that act to provide a given functional movement). This is often the case in the distal-most portions of peripheral nerve trunks (22). In the proximal portion of a nerve trunk, inter-mixing of bundles results in a more homogeneous arrangement of axons with respect to their innervation.

Sunderland has published extremely detailed investigations of the intraneural topographies of the human sciatic nerve and its popliteal divisions (23) and the human radial, median and ulnar nerves (12). These studies and others make it clear that peripheral nerve implant sites exist where nerve fascicles are not highly intermixed. Thus, selective stimulation of a fascicle can result in activation of one muscle (or muscle group). A number of selective stimulation experimental studies using epineural (24,25), intraneural or intrafascicular (26,27), or nerve cuff (6,28-33) electrode systems have in fact been reported (34).

We have recently discovered that the raccoon is an appropriate and intriguing animal model for neuroprosthetic development as it appears to be the most dexterous nonprimate model available (14,35). Known limitations of this model do include a divided median nerve in the upper arm (as opposed to a single nerve trunk in the human), limited thumb movement, and tendons that are grouped in the wrist and paw including flexor digitorium superficialis, flexor digitorum profundus, and palmaris longus. However, limb movements such as pronation, flexion of all of the digits together, and flexion of the wrist are responses suitable for evaluation of stimulation electrode technologies eventually intended for human use.

In the present study, an electrode system consisting of twelve small platinum dot electrodes imbedded in a spiral silicone rubber insulating cuff was used for selective (regional) stimulation of the median nerve of the raccoon. Snugly-fitting spiral shaped nerve cuffs enable direct apposition of electrodes to the nerve trunk. When longitudinally-aligned cuff electrode tripoles are utilized, selectivity of stimulation (i.e., restriction of the stimulating field to a local region directly under the electrodes) can be quite impressive (6,28,33). Field steering, a stimulation technique whereby sub-threshold transverse cuff electrode currents are introduced to further restrict the (stimulating) longitudinal currents into a more focused region of the nerve trunk, can be added as necessary (32). In our present study, gross movements in response to stimulation with longitudinal currents (as well as steering currents) were evaluated to assess if the cuff implant could produce many of the desired functional paw (wrist and/or digit) movements in the raccoon. Selectivity of the cuff stimulation was further evaluated by recording isometric force responses of volar forearm muscles.

3.3.2 Methods for Selective Stimulation on the Raccoon Median Nerve with Cuff Electrodes

Nerve Cuff Electrodes. Fabrication methods for multielectrode nerve cuffs similar to those used in this study have been previously reported in detail elsewhere (6). Briefly, twelve electrodes for each cuff are made by spot-welding one mm square platinum foil dots to leads of multi-strand stainless steel wire (Cooner AS631 Teflon coated). The insulating spiral-shaped cuff is made using a slightly modified method originally reported by Naples *et al.* (29) wherein two thin sheets of medical-grade silicone rubber (one stretched and one unstretched) are bonded together using uncured silicone rubber elastomer as an adhesive. The extent of stretch on the (inner) sheeting in part dictates the final resting inner diameter of the spiral-shaped cuff that results. In the actual manufacturing process, the array of twelve electrodes (configured in the form of four longitudinal tripoles separated circumferentially by a 90° spacing) with attached lead wires is first placed on the surface of the stretched sheeting. A layer of uncured elastomer is then spread over the electrodes and stretched sheeting. The unstretched sheeting is then laid on the surface of the uncured elastomer. The composite (sheeting - electrodes - elastomer - sheeting) is subsequently clamped between two highly polished stainless steel plates (with a shimmed separation distance equal to the desired final thickness of the cuff) and placed within a laboratory oven at approximately 100°C for at least 30 minutes. Such heating cures the liquid elastomer layer, bonding the layers of the cuff together. The plates are then separated, and the nerve cuff electrode insulation is trimmed to the final desired dimensions using a scalpel (see Fig. 3.3). Windows in the silicone rubber insulation are also cut in the inner surface of the nerve cuff to expose the conducting surfaces of the twelve dot electrodes.

Nerve cuff stimulation methods used were similar to those previously reported by Sweeney *et al.* (6). Briefly, for simple longitudinal tripolar stimulation, the end electrodes of three (longitudinally) aligned contacts were used as (electrically shorted) anodes and the center electrode as a single cathode. For tripolar stimulation with field steering, an additional (transverse) current was passed from the center electrode (acting as a third anode) opposite the cathode. In this situation, the total current flowing into the cathode consisted of the longitudinal current level plus the field steering current level. The transverse current level used for field

steering was generally set at a just sub-threshold level (about 20 to 30% below threshold). In this way, the field steering current level did not elicit any contractions in and of itself.

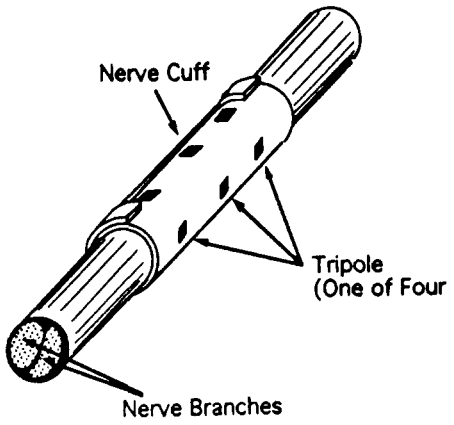

Figure 3.3 Schematic of the multielectrode nerve cuff (not to scale). Twelve platinum dot electrodes were placed on the inner surface of the nerve cuff, which contained the two median nerve cord branches.

Experimental Methods: Raccoons (Hummel Creek Kennels, St. Louis, MO) weighing 4 to 6 Kg were anesthetized with 20 mg Ketamine hydrochloride/Kg and 2 mg Xylazine/Kg body weight, intubated, and given periodic maintenance doses for anesthesia. The median nerve is divided in the upper arm of raccoons, consisting of deep and superficial cord branches (35). These two branches were exposed and electrically stimulated at first using a simple bipolar probe. The two median nerve cords were then placed together and a 'snugly-fitting' multielectrode nerve cuff was applied around them.

Forearm movements to stimulation with the cuff electrodes were observed. The animal was placed on its side with the forearm and paw free to move. A background grid (millimeter divisions) was used to help describe the movements of the forearm in response to stimulation. Using repeated stimulation, either single pulses or trains of 20 pps, stimulating currents that gave threshold, 1/2 maximal and maximal movements were determined for longitudinal and transverse currents. This was followed by the simultaneous application of longitudinal and 'field steering' (transverse) currents. The steering current was kept constant a value just below threshold for the transverse current alone and the longitudinal currents were again varied to obtain threshold, 1/2 maximal and maximal responses. Stimulation was applied with a two channel Grass (Quincy, MA) isolated and constant current stimulator (Grass Model S11 with SIU7). All studies were conducted using 10 μs pulses.

The tendons of the forearm muscles were isolated and tied with braided umbilical string having minimal stretch. Each string was tied to a force transducers (Grass) approximately 15 cm away. The force transducers could be moved along a rod to adjust resting tension to approximately 15 grams which was maintained throughout the study. Isometric forces were recorded by placing a stable pin through the elbow and clamping the paw to the table top. In addition, force transducers were clamped to stiff rods. The forces were recorded from pronator teres (PT), flexor carpi radialis (FCR), palmaris longus (PL), flexor digitorum superficialis (FDS) and flexor digitorum profundus (FDP). Recruitment curves were obtained for each experiment. Pulses of 10 μs duration again were used and forces were recorded in response to longitudinal currents followed by longitudinal currents with a 'field steering' current set below threshold for activation of the nerve with transverse (steering) currents alone. Stimulating pulse trains were 20 pulses per second (pps), applied for 1 sec. One minute was allowed between each contraction. Isometric forces were displayed on a strip chart recorder (Astro-med, West Warwick, RI).

Dissection and nerve trunk mapping were conducted to identify the relationship between the cuff electrodes and the underlying nerve fascicles. The fascicles were initially traced from the muscles to the cuff and a rough drawing of their location in the nerve was made. Sections of the nerve adjacent to the cuff were evaluated histologically to clarify the relationship of the cuff electrodes and the nerves and are presented in Fig. 3.4. Following the experiment of Raccoon #2 it was unclear whether the cuff electrode had been implanted so that the 90° was in the lateral position, and the 270° was medial, or vice versa. This matter was clarified by considering the functional responses of Table 3.4 and the recruitment responses of Figs. 3.5 through 3.7.

3.3.3 Results of Multielectrode Nerve Cuff Stimulation Studies

Anatomy of Innervation Relative to Nerve Cuff Electrodes. The arrangements of the combined median nerve cords in relation to the stimulating cuff are shown in Fig. 3.4 for all four raccoons studied. Fascicles observed in the deep median cord innervated palmaris longus (PL), flexor digitorum superficialis (FDS), and flexor digitorum profundus (FDP) (along with a branch of flexor carpi radialis (FCR) in one raccoon). There were also a large number of fascicles supplying motor and sensory innervation to the paw. The small and superficial median nerve cord was located in the ulnar direction with respect to the deep cord. Pronator teres (PT) and FCR

innervation were observed within the superficial cord along with a cutaneous branch and a coracobrachialis (CR) or biceps branch.

The approximate locations of the stimulating electrodes in relation to the nerve fascicles are also shown in Fig. 3.4. Although no intentional circumferential electrode placement was used, zero degrees was usually in the dorsal direction between the two cord branches with 90° and subsequent electrodes located in a counterclockwise direction. In Raccoon #3, the electrodes were shifted in the clock-wise direction by about 90°. It should be noted that electrode placement relative to each nerve trunk is quite approximate, given that positioning was estimated only by placing sutures into the epineurium post-mortem (to mark the estimated position of one or more tripoles). Also, as discussed earlier (see Fig. 3.3), each circumferential electrode location in actuality represents a tripolar electrode combination located longitudinally along the nerve trunk (i.e., center cathode surrounded by two adjacent anodes).

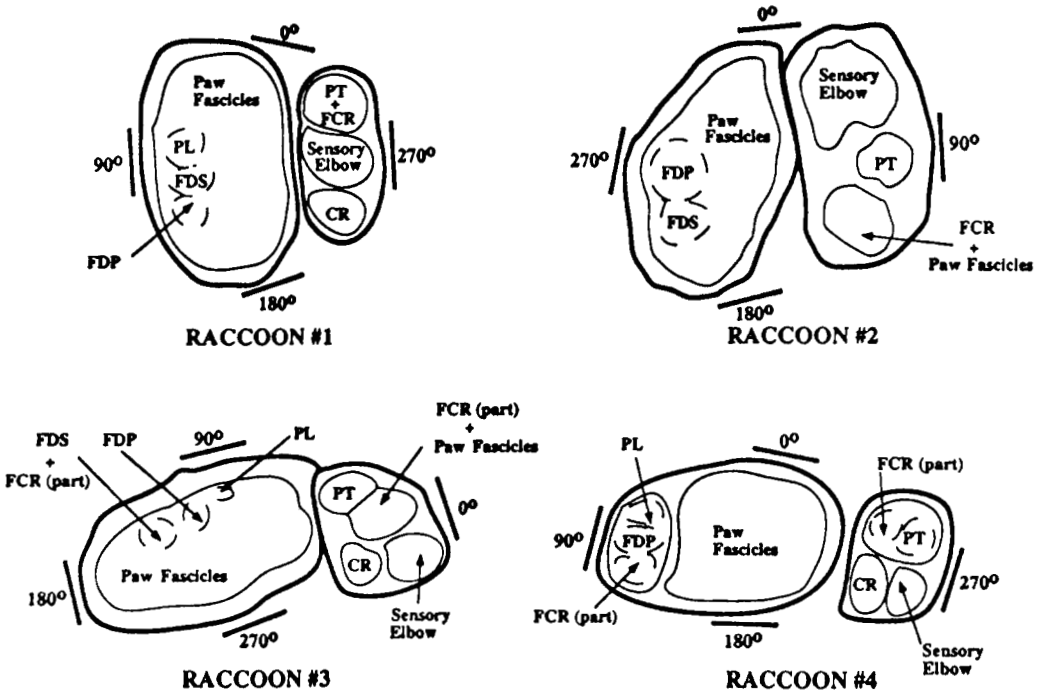

Figure 3.4 Approximate locations of cuff electrodes relative to the two cords of the median nerve. Palmaris longus (PL), flexor digitorum superficialis (FDS), flexor digitorum profundus (FDP), flexor carpi radialis (FCR), pronator teres (PT), paw and intrinsics innervation, a sensory branch and a coracobrachialis (CR) or biceps branch were noted when visible.

Forearm and Paw Movements in Response to Stimulation. Movements of the volar forearm due to nerve cuff stimulation often showed differing, selective responses depending on the (circumferential) tripolar electrode location. The threshold and other currents were evaluated for both single pulses and one second trains of pulses. The movements elicited were noted and quantified. Careful choice of electrode configuration (longitudinal tripole with or without field steering, or transverse stimulation) could often produce a highly selective functional movement (such as pronation, wrist and/or digit flexion, elbow flexion, or thumb abduction). At half and maximal stimulation, additional movements such as elbow flexion were often noted. Transverse electrode stimulation (using the center electrode of a tripole as a cathode with the opposite center electrode as an anode) at threshold often gave the same response as longitudinal (tripolar) currents although at 0.5 to 3 times larger current levels (compared to thresholds for longitudinal currents) -- although in several instances transverse stimulation produced differing responses at threshold that could be useful. High current levels were typically associated with a non-selective combination of movements. By applying both the transverse and longitudinal currents at the same time, as would be expected, the thresholds for longitudinal currents were typically much lower. The lowest available longitudinal current with our stimulation system was 0.1 mA and this low current often immediately induced a movement (indicating that the actual threshold current was even lower). Movements using longitudinal current in combination with steering currents were often similar to those seen given the longitudinal currents alone, indicating the inherent selectivity of tripolar stimulation. In some instances, as noted below, the use of steering currents clearly improved the resultant functional selectivity.

Considering Table 3.4 in detail, we see that for three of the four raccoons, a tripole could be chosen where threshold longitudinal tripole current induced pronation only -- in Raccoons #1, #2, and #3 at the 0° position (also at the 180° position in Raccoon #1). In Raccoon #4, threshold longitudinal tripole stimulation with field steering (at the 270° position) elicited a pronation response in combination with elbow flexion. In Raccoon #1 (at the 90° position with longitudinal or transverse stimulation) and Raccoon #4 (at the 0° position with transverse stimulation only, or at the 90° position with longitudinal stimulation) digit flexion could be elicited alone. A tripole configuration sometimes existed where combined wrist and digit flexion could be elicited at threshold. This was the case in Raccoon #2 (at the 90° position) and in Raccoon #3 (at the 90°

or the 180° positions). Wrist flexion alone at threshold could be elicited in Raccoon #3 (at the 90° or the 180° positions with transverse stimulation) and Raccoon #4 (at the 180° position). Elbow flexion alone could be elicited in Raccoon #1 (at the 270° position), in Raccoon #2 (at the 180° or the 270° positions with longitudinal stimulation), in Raccoon #3 (at the 270° position), and Raccoon #4 (at the 270° position). Selective thumb abduction was noted only in Raccoon #4 (at the 0, 90, and 180° positions given various combinations of longitudinal or transverse stimulation).

Tendon Force Responses To Stimulation. To further evaluate the selective stimulation performance of the multielectrode nerve cuff, the volar forearm muscles were sectioned at their tendinous insertion and attached to transducers for recording the force of contraction. Stimulation was applied for one second intervals at 20 pps and isometric recordings were obtained. Longitudinal currents were applied with and without a transverse steering current. Records from Raccoon #1 are not shown as only three muscles were recorded from. Figure 3.5 shows peak responses for Raccoon #2 using longitudinal currents (with and without steering currents). With longitudinal currents alone, recruitment of PT and FCR (followed by PL and FDP at higher currents) occurred at 0° in this animal. At 90° FDP and PL were recruited first, followed by PT and FCR. At 180° recruitment was relatively non-selective, with FDP, PL, FCR, and FCR all showing significant responses. At 270° FCR and PT were mainly recruited. The primary effect of steering current addition in this animal was to reduce the threshold level for longitudinal current stimulation. Only relatively non-significant differences in recruitment order or magnitude were seen (comparing recruitment with and without field steering).

Figure 3.6 shows similar responses from Raccoon #3. This animal did not have a strong recruitment of FCR for any of the electrodes. PT was recruited first at the 0° and 270° positions. Interestingly, the addition of field steering current with either of these configurations shifted all recruitment curves other than PT to higher threshold current levels. At the 90° position, PL was recruited first (at about 0.1 mA) followed by PT, FDP, and FDS (at about 0.2 mA). The addition of field steering current produced an interesting effect whereby the recruitment curves for PL, FDP, and FDS became separated from the curve for PT. Recruitment at the 180° position was relatively non-selective with or without field steering.

Table 3.4 Functional responses of the volar forearm to cuff electrode stimulation.

Electrode Location	Threshold (mA)	1/2 Maximal (mA)	Maximal (mA)
Raccoon #1			
0°			
longitudinal	pronate, 0.6	NA	NA
transverse	pronate, 0.9	NA	NA
steering	pronate, 0.2	NA	NA
90°			
longitudinal	digit, 0.5	NA	NA
transverse	digit, 0.7	NA	NA
steering	digit, 0.1	NA	NA
180°			
longitudinal	pronate, 0.7	NA	NA
transverse	pronate, 0.6	NA	NA
steering	pronate, 0.1	NA	NA
270°			
longitudinal	elbow, 0.5	NA	NA
transverse	elbow, 1.4	NA	NA
steering	elbow, 0.1	NA	NA
Raccoon #2			
0°			
longitudinal	pronate, 0.4	pronate, 0.7	elbow, pronate, wrist, 1.3
transverse	pronate, 1.0	pronate*, wrist, 2.5	elbow*, pronate*, wrist, 5
steering	pronate, 0.1	pronate*, wrist, 0.6	elbow, pronate, wrist, 1.4
90°			
longitudinal	wrist, digit, 0.8	wrist, digit, 1.0	wrist, digit, 1.5
transverse	elbow, wrist, digit, 1.5	elbow, wrist, 3.0	elbow, wrist, 5.0
steering	wrist, digit, 0.2	wrist, digit, 0.7	wrist, digit, 1.1
180°			
longitudinal	elbow, 0.8	elbow, 1.1	elbow, 1.5
transverse	pronate, 3	elbow*, pronate, 4.5	elbow, pronate, 6
steering	elbow, 0.4	elbow, 0.7	elbow, 1.1
270°			
longitudinal	elbow, 0.3	elbow, 0.7	elbow, 1.1
transverse	elbow, 0.6	elbow, 1.1	elbow, 1.5
steering	elbow, 0.1	elbow, 0.5	elbow, 1.0
Raccoon #3			
0°			
longitudinal	pronate, 0.2	elbow, wrist, 0.7	elbow, wrist*, digit, 1.4
transverse	pronate, wrist, 0.4	pronate*, wrist, 0.8	pronate, wrist, 1.5
steering	pronate <0.1	elbow, pronate, wrist, 0.7	elbow, pronate, wrist, 1.3
90°			
longitudinal	wrist, digits, 0.2	elbow, wrist*, 0.6	elbow, wrist*, digit, 1.1
transverse	wrist, 0.6	wrist, 1	wrist, 2
steering	wrist, digit <0.1	wrist, 0.5	wrist, digit, 0.2

Table 3.4 (continued)

Electrode Location	Threshold (mA)	1/2 Maximal (mA)	Maximal (mA)
180°			
longitudinal	wrist, digit, 0.2	wrist, digit, 0.5	elbow, wrist, digit, 0.8
transverse	wrist, 0.4	wrist, 0.9	wrist, 1.2
steering	wrist <0.1	wrist, 0.5	elbow, wrist, 1.5
270°			
longitudinal	elbow, 0.3	elbow, wrist, 0.7	elbow, wrist, 1.5
transverse	elbow, 1	elbow, wrist, 2	elbow, wrist, 4
steering	elbow, wrist <0.1	elbow*, wrist, 0.4	elbow*, wrist, 1
Raccoon #4			
0°			
longitudinal	thb abd, 0.8	pronate, wrist, thb abd, 1.6	pronate, wrist, digit, 3
transverse	digit, 1.1	elbow, wrist, digit, 3	elbow, wrist, digit, 6
steering	thb abd, 0.1	wrist, digit, 0.8	wrist, digit, 1.4
90°			
longitudinal	digit, 0.4	wrist, digit, 0.9	wrist, digit, 2
transverse	thb abd, 0.8	elbow, wrist, digit. 1.2	elbow, wrist, 3
steering	thb abd, 0.1	wrist, digit, 0.8	wrist, digit, 1.5
180°			
longitudinal	wrist, 0.3	wrist, digit*, 0.9	elbow, wrist, digit*, 2
transverse	thb abd, digit, 0.9	wrist, digit, 2.5	elbow, wrist, digit, 6
steering	thb abd, 0.1	wrist, digit, 0.7	wrist, digit, 1.5
270°			
longitudinal	elbow, 0.3	elbow, pronate, wrist, 0.6	elbow, wrist, pronate, 1.2
transverse	elbow, 0.5	elbow, wrist, 0.6	elbow, wrist, pronate, 1.5
steering	elbow*, pronate, 0.1	elbow, pronate, wrist, 0.6	elbow, pronate, wrist, 1.3

While observing the paw, a threshold response was just perceptible; the maximal response was noted when further increases in current did not appear to induce further increases in paw or forelimb movements and the half maximal response was estimated as a current approximately half way between the threshold and maximal response.
*most prominent response.

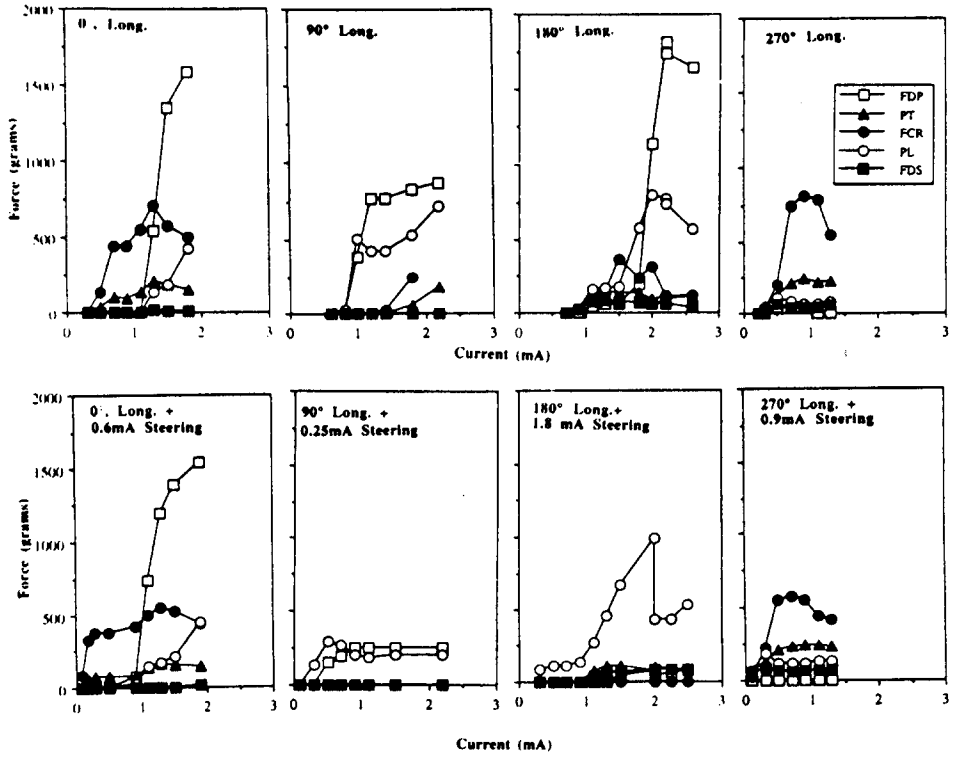

Figure 3.5 Tendon force responses to stimulation with longitudinal current (with and without field steering) using electrodes at the 0, 90, 180 and 270° positions for Raccoon #2.

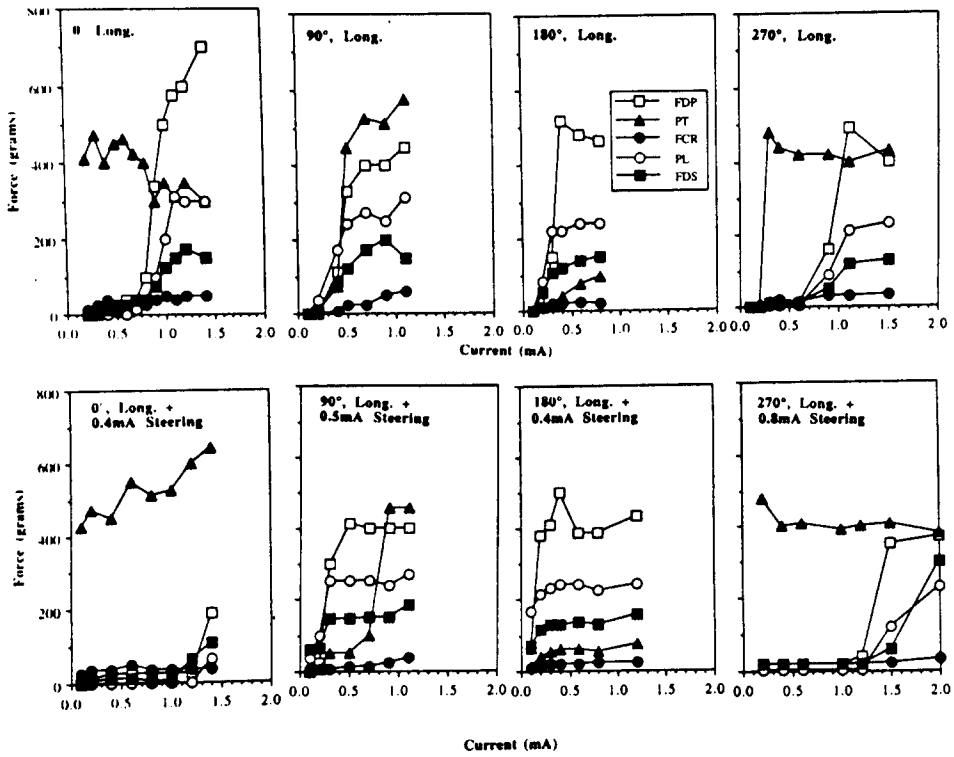

Figure 3.6. Tendon force responses to stimulation with longitudinal current (with and without field steering) using electrodes at the 0, 90, 180 and 270° positions for Raccoon #3.

Raccoon #4 response are depicted in Fig. 3.7. Recruitment at the 0° position was relatively non-selective, with FDP, FDS, and PL showing slightly lower thresholds than FCR and PT. Longitudinal stimulation at the 90° position without field steering yielded recruitment of FDP, PL, and FDS at a threshold of about 0.5 mA -- followed by FCR and PT at 1 to 1.5 mA. Stimulation with field steering at this position reduced the threshold for FDP and PL recruitment to about 0.1 mA. -- while the thresholds for FCR and PT were relatively unaffected. Recruitment at the 180° position without field steering was relatively non-selective. Addition of field steering to this configuration did provide more selective activation of FDP. FCR was activated (along with some FDS response) first at the 270° position. Field steering at 270° reduced the thresholds for FCR and FDS with little effect on the thresholds of the other muscles.

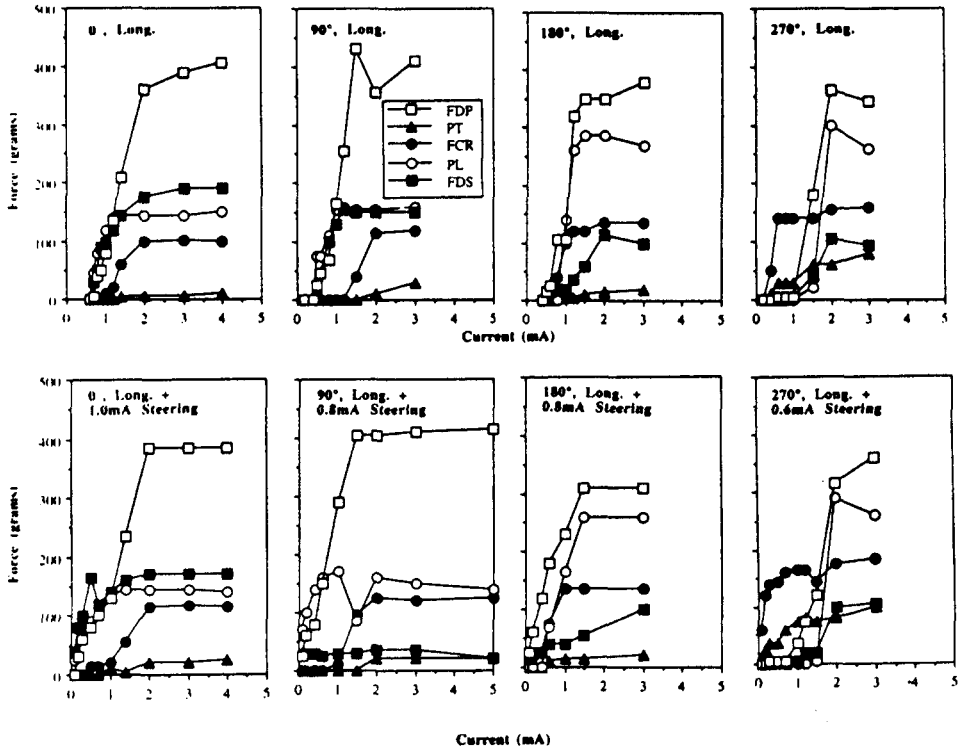

Figure 3.7. Tendon force responses to stimulation with longitudinal current (with and without field steering) using electrodes at the 0, 90, 180 and 270° positions for Raccoon #4.

Electrode Configuration and Stimulation for Producing Selective Responses. The fascicular maps of Fig. 3.4, along with the functional response data of Table 3.4 and the recruitment curve data of Figs. 3.5-7, can be considered in combination to describe choices of electrode configuration and stimulation application which would be best for producing a desired selective response in each experiment. That is, which tripole (0, 90, 180, or 270°) and which stimulation strategy (longitudinal, transverse, or longitudinal with field steering) would be chosen to yield a functional movement (e.g., digit, wrist, digit & wrist, or elbow flexion; or pronation)? Table 3.5 lists the possible tripole choice, stimulation approach, current level, and probable muscle(s) activated for selective threshold responses. Muscle(s) are listed based on recruitment data and/or fascicular mapping. Note that elbow flexion, when seen, was probably due to recruitment of muscles such as the coracobrachialis ('CR', which was sometimes mapped but was not recorded isometrically).

3.3.4 Discussion of Multielectrode Nerve Cuff Stimulation Studies.

We have shown selective activation of the volar forearm and paw to median nerve activation with a twelve electrode cuff. Major movements such as pronation, wrist flexion, and digit flexion were observed. The greatest selectivity was observed at threshold currents. In all four raccoons studied, a threshold electrode choice and stimulation strategy could be identified that enabled selective production of either digit flexion, wrist flexion, and/or digit and wrist flexion. However, only in one animal (#4) could any of these three responses be chosen individually (see Table 3.5). It was possible to elicit a selective pronation response at threshold in three of the four animals. Selective elbow flexion at threshold could be produced in all four experiments. At higher currents additional movements were usually induced. This result could actually be of benefit if combined movements (e.g., elbow, wrist, and digit flexion, etc.) were desired. Steering current primarily changed the stimulation threshold and in some instances acted to separate recruitment curves in a beneficial manner. In one animal (#3) the use of field steering produced a selective threshold response (wrist flexion) which could not be obtained with simple longitudinal tripolar current. Such results are reasonably consistent with previous multielectrode stimulation studies (6,28,33,36). Multielectrode cuff electrode systems generally rely upon an ability to 'focus' an excitatory electric field in a small sub-region of a nerve trunk beneath the center electrode of a

longitudinally aligned tripole. Highly selective, functional responses can only be obtained when fascicles can be activated in unison (or in functional groups). Clearly, despite the fact that the raccoon median nerves have complex, multi-fascicular structures, reasonably selective functional responses can be elicited at threshold using multielectrode nerve cuffs. It is possible that use of more elaborate combinations of electrodes (6) or non-standard waveforms (37) could yield even greater selectivity. The raccoon digit and forearm pronation movements may be re extensive than other animals such as the rat, cat or dog. The raccoon therefore appears to be a suitable, if challenging, animal model for further development of not only long-term implantable nerve cuff electrode approaches (14) but perhaps other stimulation electrode technologies prior to human neuroprosthetic studies.

Table 3.5. Electrode and stimulation choices for producing responses.

Digit Flexion	Wrist Flexion	Digit & Wrist Flexion	Pronation	Elbow Flexion
Raccoon #1				
90°	Not observed	Not observed	0°	270°
Longitudinal			Longitudinal	Longitudinal
0.5 mA			0.6 mA	0.5 mA
PL; FDS; FDP			PT; FCR	CR
Raccoon #2				
Not observed	Not observed	90°	0°	270°
		Longitudinal	Longitudinal	Longitudinal
		0.8 mA	0.4 mA	0.3 mA
		PL; FDP	FCR; PT	PT; CR
Raccoon #3				
Not observed	180°	90° or 180°	0°	270°
	Field steering	Longitudinal	Longitudinal	Longitudinal
	<0.1 mA	0.2 mA	0.2 mA	0.3 mA
	PL; FDS; FDP	PL; FDP; FDS	PT	CR
Raccoon #4				
90°	180°	90° or 180°	Not observed	270°
Longitudinal	Longitudinal	Longitudinal*		Longitudinal
0.4 mA	0.3 mA	0.9 mA		0.3 mA
FDP; PL; FDS	FCR?	FCR;FDP;PL;FDS		CR

All responses at threshold except for the digit and wrist flexion response of Raccoon #4 (noted by *) which could be elicited with half-maximal longitudinal currents.

3.4 The Raccoon as an Animal Model for Testing Somatosensory Neuroprostheses

Study conducted by Charles J. Robinson, D.Sc., P.E., Departments of Rehabilitation Science and Technology, Electrical Engineering and Orthopedic Surgery, The University of Pittsburgh, Pittsburgh, PA, USA, and Rehabilitative Neuroscience Laboratory, Physical Medicine and Rehabilitation Service, 117 Highland Drive, VA Medical Center, Pittsburgh, PA USA; Robert Wurster, Ph.D., Departments of Physiology and Neurosurgery, Loyola University Stritch School of Medicine, Maywood, IL, USA, and James S. Walter, Ph.D. Rehabilitation R&D Center (151L), Hines VA Hospital, Hines, IL USA, and Department of Urological Surgery, Loyola University Stritch School of Medicine, Maywood, IL, USA.

3.4.1 Somatosensory Neuroprostheses

With respect to somatothesis, the raccoon ranks with human and non-human primates in the use of the hands and in the proportional amount of cortex devoted to the representation of the digits. However the cortical neuroanatomy of the raccoon is unique in that the areas where each digit is represented are identifiable via gross cortical landmarks around the tri-radiate sulcus (38) and quite easily separable during neurophysiological recording. Individual sub-gyri are present, one for each digit, that provide landmarks for electrode placement. Thus, peripheral stimulation of a digit either by light touch or by fine-wire electrical stimulation will produce a response in a location within the raccoon's contralateral somatosensory cortex that can be readily distinguished from the locations that respond to stimulation of the other digits.

The precision of this mapping prompted us to investigate the centripedal effects of stimulating the median nerve with a nerve cuff electrode that permits stimulation of selected quadrants of the nerve. We had previously studied the chronic effects of implantation of the cuff (14) and the selectivity of the cuff in producing movements of the raccoon forelimb, wrist and digits (39,40). This 12-electrode cuff has 4 sets of longitudinally aligned platinum-dot tripolar electrodes arranged in each quadrant of the cuff (i.e., in 90° steps). More details of the cuff and the evolution of its design can be found in references 2 through 8.

We (39,40) and others (6,28,32,33) had already demonstrated that selective stimulation with such a cuff can produce discrete and separable movements in the muscles innervated by the median nerve, and that muscle force can be graded by increasing the stimulus intensity. We wondered whether a similar separation could be found within that part of the somatosensory cortex that is activated from the median nerve. If such a selective activation were to be found, it could have immediate clinical application in the restoration of the tactile sense to individuals with upper limb amputation. The signals from artificial tactile sensors could be used to control the magnitude and location of stimulation to the residual nerve trunk.

3.4.2 Methods for Evaluation of the Raccoon Model in Somatosensory Neuroprothesis Research

Animal Preparation. As a pilot study, these cortical mapping experiments were carried out immediately after the movement-response studies described in our other papers (39,40) were finished (Raccoon #1), or after assessment of the stability of motor responses following 6 weeks of chronic implantation of a different style cuff (41) on the right forelimb (Raccoons #2 and #3). Thus the experiments described here were carried out with each raccoon deeply anesthetized and respirated. Ketamine hydrochloride (20 mg/kg) and xylazine (2 mg/kg) were used as initial induction agents for all three animals. The raccoons were then maintained on pentobarbital anesthesia (P.R.N. intraperitoneally). These raccoons (Procyon Lotor) were bred for research (Hummal Creek Kennels, St. Louis, MO) and weighed 4 to 6 kg. At the conclusion of the experiment, the animals were overdosed with pentobarbital, injected intracardially with KCl, and removed from respiratory assist.

For Raccoon #1, a 12-electrode nerve cuff had previously been implanted around the median and radial nerves in the left upper arm for movement studies (40). Videographic recordings had been made of the discrete muscle movement produced by selective cuff stimulation. Then the forearm down through the middle of the palm was dissected to permit direct force recordings from the tendons of the extrinsic muscles of the paw and wrist. The thenar and hypothenar eminencies and all of the hairy and glabrous surfaces of the digits were left intact, so that a cortical mapping study could still be carried out immediately after these other studies. The results from these two previous experiments are compared in Section 3.3.4 with the results of this mapping. As part of

the protocol of the companion experiment, a post-mortem dissection of the median nerve innervation pattern to specific muscles of the paw and forearm was carried out. The results of this dissection were then related to the orientation of the nerve branches in the cuff (see Fig. 3.8).

This study deals primarily with Raccoon #1, since motor (both gross and individual tendon), sensory and innervation data were available. Raccoons #2 and #3 were part of another protocol, where a four-electrode nerve cuff had been implanted 6 weeks previously around the deep and superficial branches of the medial nerve and the radial nerve in the right upper arm to test the chronic stability of the electrode described in reference 41. No tendon dissection was carried out in these latter animals, thus the distal peripheral forearm remained intact. After testing for motor responses, this cuff was resected along with the nerves that it encircled (with an additional 5 mm taken of the nerves as they exited both sides of the cuff).

These two raccoons were then used to verify that the 12-element recording array could demonstrate selectivity in another raccoon. In these raccoons, the median nerve was located in opposite (left) forearm by dissection just above the elbow. The 12-electrode cuff was acutely placed over the two branches of the median nerve as previously described (40). No post-mortem analysis of innervation pattern or of the orientation of the nerve within the cuff was carried out for these raccoons.

For these cortical mapping studies, each raccoon was placed in a stereotaxic frame (David Kopf) to provide stability. A craniotomy was performed to allow access to the raccoon somatosensory area of the right hemisphere. This oval-shaped craniotomy extended from the frontal sinus anteriorly, to within 5 mm medially of the midline, to a line 2 to 2.5 cm lateral to the midline and parallel to and just adjacent to the zygomatic arch (requiring bisection and elevation of the temporalis muscle), to about 4 to 6 cm posterior of the bregma. The somatosensory area was centered in this opening, on the third major gyrus back. The dura was reflected to remove it from that region. The stereotaxic frame was rotated to the left so that opening was roughly horizontal to aid in keeping the cortex moist via repeated lavages with physiological saline. A sketch (Raccoon #1) or digitally captured photograph (Raccoons #2 and #3) of the representative

cortical landmarks (i.e., blood vessels, sulci, and gyri) was used to record the placement of the recording electrode and for comparison with previous published maps (38).

Recording and stimulating techniques. A bipolar surface-recording electrode was constructed from two platinum-tipped metal microelectrodes, suitable for mass potential recording, that were separated by 2 to 4 mm. The electrode was positioned on the surface of the cortex using a Kopf stereotaxic three-axis electrode carrier tilted to be perpendicular to the surface of the cortex. The electrodes were differentially connected to a Grass 511J AC amplifier through a Grass HIP511E high impedance preamplifier. A reference recording ground was clipped to the ear or tongue bar of the stereotaxic frame. Filters were set at 30 to 3000 Hz, with the 60 Hz notch filter engaged. The output of the amplifier was displayed on a Gould digital storage oscilloscope (Model 4162), that could be triggered by the stimulus, and that could plot captured waveforms of interest. The output of the Grass amplifier was fed to an audio amplifier to provide auditory feedback as an aid to localizing the peripheral receptive field of the recording location. This peripheral field was determined by localized tactile stimulation of the raccoon hand and noted on a diagram of the hand.

The 12-electrode nerve cuff has been well described elsewhere (6,28,32,33,40). It consists of 4 longitudinal rows of three 1 mm platinum 'dots', with each row located 90° apart (Fig. 3.8). The middle dot of each row serves as the stimulation cathode, while the two outer dots of each row are electrically connected and serve as the anode. A 'steering current' could also be employed by additionally anodically stimulating the center electrode located 180° from the cathodic electrode. The electrode dots were placed on an elastomer material that caused the assembly to curl into a spiral tube (29) that was then placed around the nerve. Each quadrant of the tripolar sets were individually stimulated, while taking note of any cortical evoked response or evoked movement. The stimulus pulse was generated by a two channel Grass constant current stimulator (Grass Model S11 with SIU7 isolator), either as a single stimulus or with a repetition rate of 1/sec. Stimulus pulse duration was 10 μsec for Raccoon #1 and 100 μsec for Raccoons #2 and #3.

The intensity of stimulation was increased until a repeatable and noticeable short-latency evoked response occurred in the cortex. The threshold for a movement response was also noted.

Stimulation was increased in a given quadrant until either gross movement ensued or the cortical response saturated. At no time did stimulation exceed 12 ma, even if an evoked response was not seen. Response latencies were determined by inspection of the oscilloscope tracings or printouts from the Gould oscilloscope.

For data analysis, the cortical receptive field locations were compared with each quadrant's stimulation threshold. For Raccoon #1 these results could also be compared with the movement profiles obtained in the previous experiment.

3.4.3 Results of Somatosensory Neuroprosthesis Study

As expected (38), when the recording electrode was placed within the digit area of each raccoon's somatosensory area, a small circumscribed peripheral receptive field could generally easily be identified on the digits or hand that was maximally sensitive to tactile stimulation. In all three of the raccoons, stimulation of each quadrant of the cuff electrode array also produced a short latency biphasic response in the digit and/or distal forelimb area of the contralateral somatic sensory cortex, and no response outside of that cortical area. However, the threshold stimulation current needed to produce a cortical evoked potential did vary between quadrants and between raccoons, as did the current to produce movement.

Raccoon #1. The best example of cortical selectivity to cuff stimulation occurred in Raccoon #1. Cortical recording Site #3 was activated from the web region between the digits (see Fig. 3.8), but also could be activated from the dorsal hairy skin of digits 3, 4 and 5 (suggesting a more radial distribution). From the nerve cuff, this site was best activated by low threshold stimulation of electrodes at 0 and 270° (both less than 0.1 ma). Threshold activation of this site through the electrodes at 90 and 180° required 6 to 15 times more current to produce an observable response. Figure 3.9 compares the responses elicited from each quadrant to a single stimulus pulse of 0.1 ma. This current level was below the movement or muscle activation thresholds of 0.2 mA (0, 90, 180°) and 0.3 mA (270°) (see reference 40).

Cortical Site #1 in Raccoon #1 was on the gyrus immediately medial to that of Site #3. It was activated by light touch to a banana-shaped receptive field that was most sensitive on the lower,

lateral part of Digit 1 but that continued across the web to the lower medial aspect of digit 2 (see Fig. 3.8). Bipolar needle stimulation of lateral digit 1 produced a response with a 14 msec latency (Fig. 3.10C). This site was best activated from the nerve cuff by low threshold stimulation of electrodes positioned at 90 and 180° where a robust response was produced (Fig. 3.10B, produced with 1 mA stimulation current)). Threshold activation of this site through the electrodes at 0 and 270° required 3 to 5 times more current to produce an observable response (Fig. 3.10A, produced with a 2 ma stimulation current). The use of a steering current through the electrode at 180° reduced the threshold for activation of the 0° electrode by 50%.

Recording Site #5 in Raccoon #1, which was on the gyrus anterior to that of Site #1, also produced a distinction in orientation responsivity that fell between that shown by Sites #1 or #3. Site #5 was activated by light touch to the medial side of the distal portion of digit 2. From the nerve cuff, this site was best activated by low threshold stimulation of electrodes positioned at 0° and 270° (0.2 mA and 0.1 mA). Threshold activation of this site through the electrodes at 90° and 180° required 2 to 4 times more current to produce an observable response (0.5 and 0.7 mA). Site #4 was on the gyrus immediately anterior to that of Site #3 and lateral to Site #5. A peripheral receptive field could not be found (but recall that part of the palm had been dissected for the tendon studies). For nerve cuff stimulation, this site behaved almost identically to that of Site #5.

Recording Site #2 was on the gyrus immediately posterior to that of Site #1. Electrical stimulation of neither the skin or the cuff produced a response, suggesting that Site #2 was posterior to the digit area of somatosensory cortex.

Raccoon #2. After initial implantation, two of the four quadrants of the stimulating cuff implanted in Raccoon #2 neither produced movement nor a cortical evoked potential (at any recording site) even with stimulating currents in the 9 to 12 mA range. Thus only the electrodes at 180° and 270° could be used for the evoked potential study. When the electrode array was removed at the end of the experiment, the electrical connections to the 0° and 90° electrodes were found to be broken.

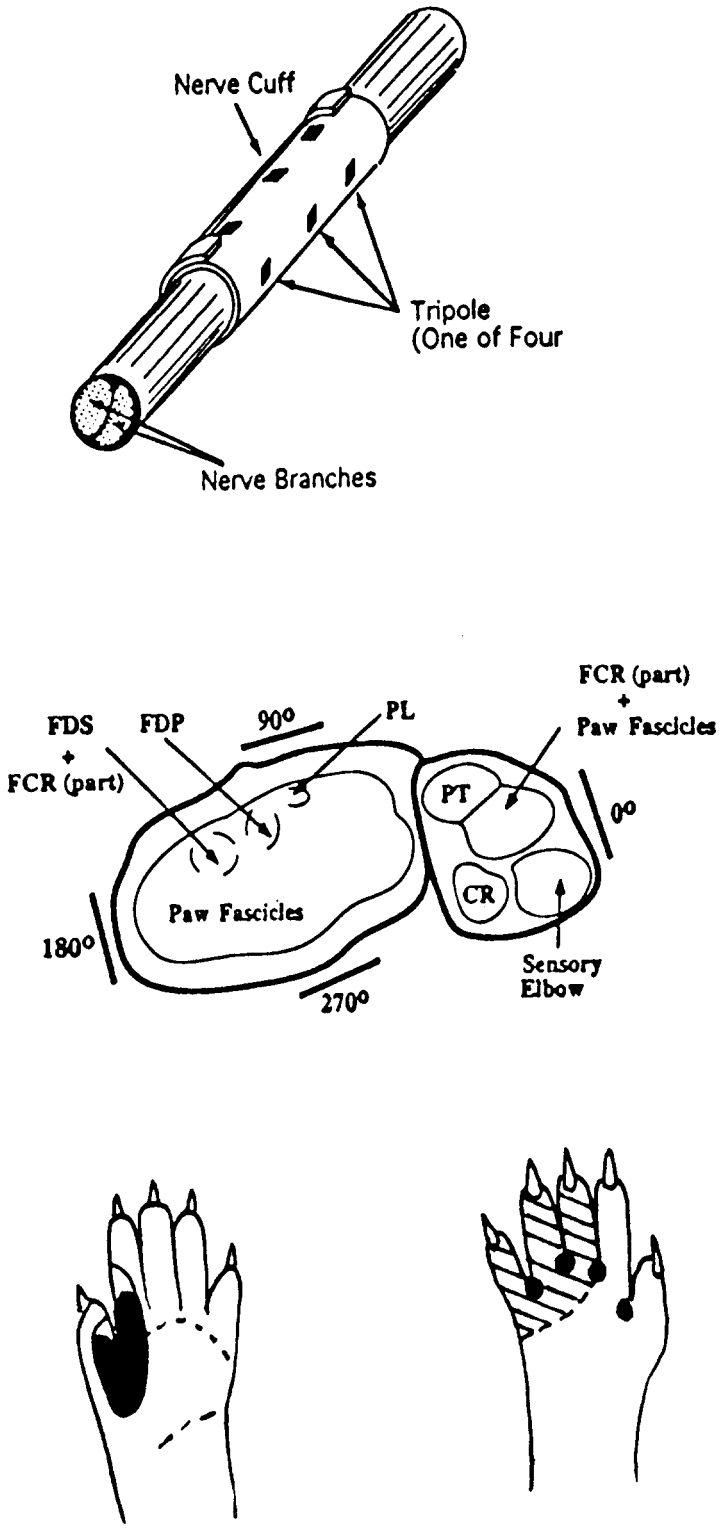

Figure 3.8 Twelve-element, tripolar nerve cuff electrode (top) and the orientation of the electrode on the two branches of the median nerve (see reference 40 for details) in Raccoon #1. The receptive fields in Raccoon #1 for cortical recording Site #1was on the glabrous skin of digits 1 and #2 (bottom left), and for cortical Site #3 in the webbing between the digits and on the hairy shin overlying digits 3, 4 and 5 (bottom right).

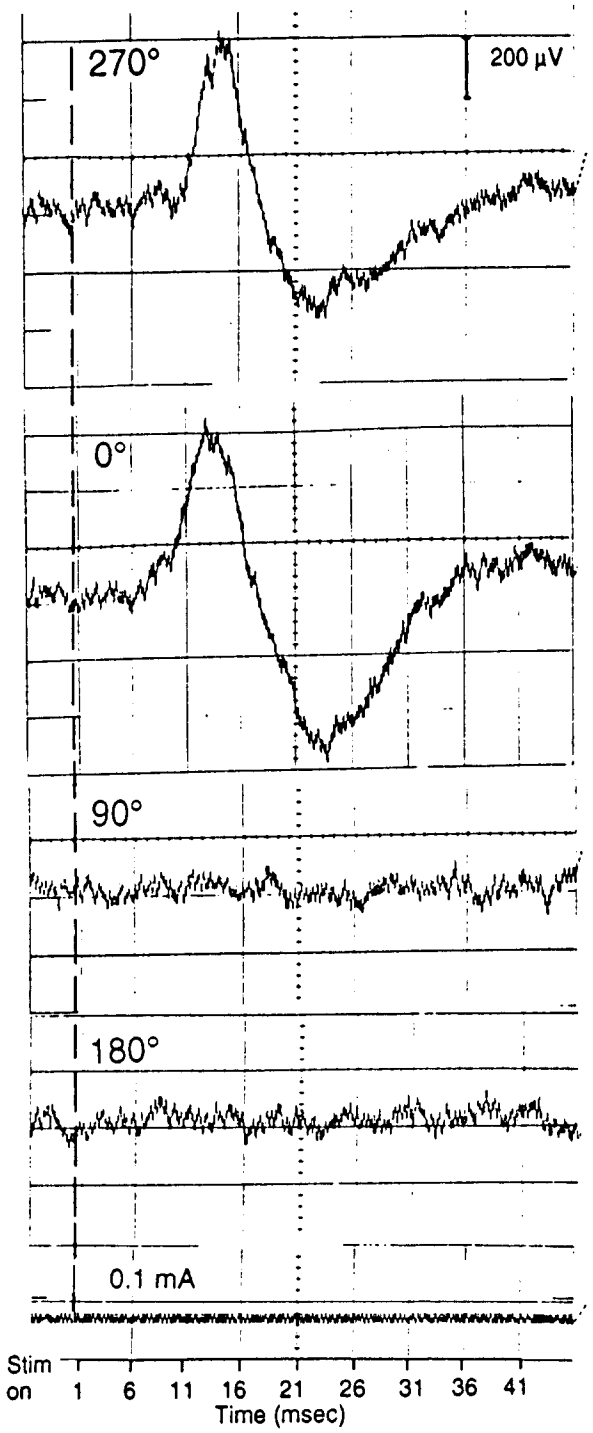

Figure 3.9 Comparison of the responses at recording Site #3 evoked by electrical stimulation of each quadrant of the cuff electrode with a single 10 µsec, 0.1 mA pulse.

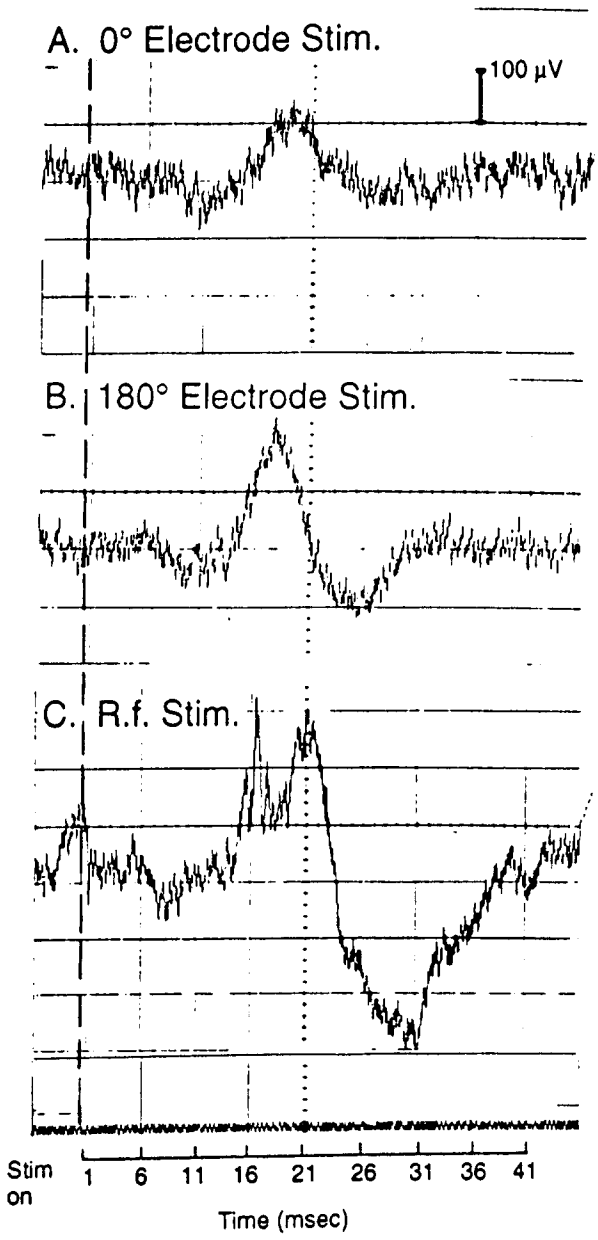

Figure 3.10 Cortical surface responses at recording Site #1 evoked by electrical stimulation of:

A) the 0° quadrant of the cuff electrode (at 2 mA);
B) the 180° quadrant of the cuff electrode (at 1 mA); and
C) the peripheral receptive field by a pair of needle electrodes (at 40 mA).

Four cortical recording locations were studied within the distal forelimb, hand and digit areas of the somatosensory cortex of Raccoon #2. Evoked potential thresholds were between 0.53 and 0.75 mA for the 270° quadrant; and between 3.0 and 5.0 mA for the for the 180° quadrant. Very discrete twitch or flexion movements of the outer phalanges of digit 1 (and occasionally digit 2 at higher currents) could be produced by stimulation of either the 270° quadrant (at thresholds ranging from 0.73 to 2.2 mA) or of the 180° quadrant (at thresholds between 4 and 5 mA). At 3 of the 4 locations, the threshold current for producing an evoked potential via the 270° quadrant was less than that to produce digit movement. In the one case where 0.75 mA produced a twitch of digit 1, the sensory receptive field was located on the glabrous skin in the middle of digit 1, and could have been (but probably was not) activated by the movement itself.

Perhaps the most intriguing aspect of the cortical recordings for this raccoon was the observation that the threshold stimulation currents needed evoke a response were equal (0.75 mA, 270° quadrant) for two recording sites separated mediolaterally from one another by over 1 cm. The more medial site was activated by the slight movement of hairs on the postaxial forearm, while the lateral site was activated from the glabrous skin of the middle portion of digit 1. The lowest evoked potential threshold seen (0.53 mA), and the largest evoked potential threshold amplitude, was recorded at a Site #2 mm from the latter site, at a locus activated from the proximal glabrous parts of digits 2 and 3 and the neighboring palm area.

Raccoon #3. Marked differences in responses to quadrant stimulation were also seen in Raccoon #3. Stimulation of either the 0° or the 270° quadrants with an 0.18 mA pulse consistently produced a rapid elbow flexion and limb withdrawal movement. The threshold current needed to produce an evoked potential when stimulating these quadrants was 0.24 and 0.30 mA, respectively. We did not pursue further stimulation of these two quadrants, since we felt that the large amount of movement produced by stimulation would confound any cortical evoked potential mapping.

In contrast, stimulation of the 180° or 90° quadrants produced a flexion twitch of digit 2 at a threshold of 0.85 mA (180°) or a flexion of the tip of digit 3 at a threshold of 1.0 mA (90°). For these two quadrants, the evoked potential current threshold was almost always below that of the

movement threshold, as long as the recordings were done within the forelimb and hand area of cortex. For the six locations studied within this area, evoked potential thresholds ranged from 0.48 to 0.75 mA for the 180° quadrant, and from 0.62 to 0.9 mA for 90°. The 180° quadrant always required slightly less current to produce an evoked potential than the 90° one.

When the recording locus was shifted far medially so that activation could no longer be achieved from the forelimb (but with a hint of peripheral activation from the foot), the threshold to produce an evoked potential exceeded that to cause a movement. And when the recording location was moved to a posterior-medial site well outside of the somatosensory cortex, no evoked potential could be elicited, even though a substantial amount of wrist flexion was being produced by the stimulation (1.5 mA to either the 90° or 180° quadrant).

3.3.4 Discussion of Somatosensory Neuroprosthesis Study

Selectivity in Peripheral Somatosensory Neuroprosthetic Coupling. The designer of a sensory neuroprosthesis has a number of options to choose from when considering the optimal location to couple a representation of the sensory signal into that sense's neuronal pathway. Coupling can be made at a peripheral location, at one of the various relay nuclei, or at the cortical level. Tradeoffs include ease of implantation, degree of invasiveness, patient safety, the type and specificity of sensory mapping that occurs at each location, and many other factors. In the neuroprosthetics field, electrical stimulation of sensory afferents for functional restoration currently finds its most accepted clinical application to be its use for cochlear implants. These implants have been shown to restore functional hearing to some of its recipients, and an awareness of sound to some others.

Neuroprosthetics for functional restoration of movement have tended to be applied in the periphery, with coupling generally near the motor end plate, on the efferent nerve or at the surface of the overlying skin (34). Suprathreshold stimulation of the entire nerve itself in this application will excite both the desired efferent fibers to the muscle, the spindle afferents from the muscle, and any A-beta (touch) cutaneous afferents that are in the nerve. Thus, if the nerve is to be the site of coupling, some means of selective activation of a specific fascicle of the nerve bundle or of a specific type of fiber within the bundle needs to be found. To achieve this specificity, a number of

individuals have proposed stimulation techniques or electrode designs (6,26-29,32,34) to activate only a portion of the nerve.

For the studies that we report here, we have chosen one of the simplest means of selective activation, that of longitudinal tripolar stimulation by quadrant, via a 12-electrode spiral nerve cuff assembly. We make no claim that these are the best electrodes to use to achieve selectivity, but claim only that selective activation has been demonstrated by their use, specifically for eliciting differentiable movement responses.

The Raccoon and Somatothesis. If such selectivity can be demonstrated on the motor (efferent) component, we wondered whether such selectivity also existed on the somatosensory (afferent) side. The raccoon, being one of the penultimate animals in terms of manual dexterity and having such a large and grossly differentiable amount of cortex devoted to the hand and digits, seemed to be a natural vehicle for testing this hypothesis. Also the cutaneous domains and the sensory organization of the nerves innervating the raccoon forepaw has been documented (42), as has the motor innervation to its forelimb and hand (39). The raccoon primary somatosensory cortical area has been well studied (see references 38, 43-45). Further, the representation of each digit can be subdivided into glabrous and heterogeneous areas illustrating that fact that afferent projections from the glabrous skin and dorsal hairy skin of each digit are largely segregated at all levels of the raccoon somatosensory system (44). This observation is of interest since Doetsch, *et al.* (43), have noted that, under alpha-chloralose anesthesia, the heterogeneous part of a digit's representation received a greater convergence (i.e., from a larger receptive field) when measured by localized electrical stimulation of the peripheral receptive field than by natural (i.e., tactile) stimulation.

In concept, then, different loci within the digit and hand area of raccoon somatosensory cortex should be activated by stimulating different quadrants of an electrode encircling the median and/or radial and/or ulnar nerves. And no activation should be seen at cortical loci outside of the innervation area represented by these nerves.

Our preliminary observations, especially in Raccoon #1, do demonstrate that selective activation can be achieved at the cortical level. In comparing recording sites #1 and #3 in this raccoon, note

that the cortical area representing the glabrous skin of digits 1 and 2 (Site #1) was activated by cuff stimulation at 90° and 180° while the area representing the hairy skin of the more lateral digits (Site #3) was best activated by cuff stimulation at 0° and 270° This observation is consistent with the positioning of the electrodes (see Fig. 3.8). One might expect the 0° electrode to have a more radial sensory distribution since the efferents to flexor carpi radialis, carpi radialis and pronator teres lie directly under the 0° electrode and adjacent to the 270° electrode, and were the muscles first activated by stimulator of these two electrodes (40). Likewise, stimulation of the electrodes at either 90° and 180° produced movement of the wrist and digits at a threshold of 0.2 mA to a 20 Hz pulse train (4).

Selectivity was not as evident in Raccoons #2 and #3 where the demonstration of a possible selectivity was greatly hampered by the fact that we could only use two of the four cuff quadrants for stimulation. Part of the reason for the difference might also be that longer stimulation pulse widths were used in the latter two raccoons (100 μsec) than in the first (10 μsec). However, there still remained appreciable differences in the current thresholds of the two usable quadrants; and these differences could be exploited to insure selectivity. It would certainly be worthwhile to repeat these experiments with electrodes like those described in references 11 and 12 that might be capable of greater selectivity. Also, the bipolar recording electrode itself, and the mass evoked potential that it records, might not be selective enough to show quantifiable differences in activation. Such quantification might require the use of electrodes capable of recording single- or multi-unit neuronal activity.

Finally, a comment needs to be made regarding the recording of cortical responses under barbituate anesthesia. Generally the signaling pathway from the somatic periphery to somatosensory cortex is very robust, with cortical neurons able to respond to rapid sequences of peripheral stimuli. Under the deep barbiturate anesthesia that we used, we were unable to entrain the evoked potentials to a 20 Hz train of 6 pulses delivered to the stimulating cuff. In general, the first pulse would evoke a response, followed by barbituate spindling that seemed to interfere with the responses to subsequent pulses. However, other anesthetic agents used in the study of raccoon cortex, such as alpha-chloralose (43), tend to overemphasize the effects of the somatic

input. Again, it would certainly be worthwhile to repeat these experiments with another type of anesthetic.

Clinical Utility and Cortical Reorganization. Since the median nerve is a mixed somatic and motor nerve, cuff stimulation can excite both afferent and efferent axons. However, we were able to demonstrate in all three raccoons that a sensory evoked potential could be produced (at least for one quadrant) at a current level below the level that produced a movement.

But the clinical utility of a somatic sensory neuroprosthesis involving a peripheral nerve might best be found in restoring the tactile sense after amputation. If the peripheral nerve were cut by the amputation, movement could not occur due to stimulation. Assuming that the afferent pathways from the nerve stump to the cortical receiving area of the denervated skin remain intact (or latent), then stimulation of the nerve above the amputation could in theory be used to provide localized cortical activation by selective stimulation. However, since reorganization of the raccoon somatosensory cortical representation occurs following selective digit amputation (45,46), it is also reasonable to assume that amputation of an entire limb would produce a more profound reorganization. It remains a testable conjecture to determine whether selective stimulation of a nerve stump could produce a new and reorganized cortical representation.

3.5 Evaluation of a Chronically Implanted Thin-Film Peripheral Nerve Cuff Electrode on the Median and Ulnar Nerves of a Raccoon

3.5.1 Introduction to Chronic Cuff Implantation Study

A key issue with FNS is nerve injury associated with the implanted electrode (24,29,47-52). In a recent report, loss of a small percentage of neurons was associated with the connecting cable and mechanical injury caused by a twisted cuff (48).

The present study evaluated nerve injury with a highly flexible thin-film cuff with cable implanted on the raccoon median and ulnar nerves. The cuff electrodes were fabricated from FEP Teflon film using a thermal forming process. The thin-film cuff was self-sizing and provided a novel circumneural electrode. An implantation period of six weeks was chosen to allow for the

development of a connective tissue sheath around the cuff and nerve (48). Nerve degeneration was evaluated histologically.

3.5.2 Methods for Chronic Evaluation

Four raccoons were anesthetized with 20 mg Ketamine hydro-chloride/Kg and 2 mg Xylazine/Kg body weight, intubated, and put on a respirator with respiratory anesthetics for our implantation procedures. Following the implantation surgery, the animals were housed for 42 days (6 weeks) in standard cages and fed a standard cat diet with water.

Cylindrical cuff electrodes with an electrode lead were used for this study. The inner diameter of the cylinder was 3.5 to 4 mm and the electrode length was 1 cm. The cuff was made of a thin film (0.5 mm) of FEP Teflon. Four electrodes were equally spaced around the middle of the cuff as shown in Fig. 2.3. The electrodes were formed by sputtering a multilayer metal film of Ti and Ir on thin sheets of the Teflon and photolithographically patterning and etching the leads and charge injection sites. The patterned substrate was then thermally sealed with a second polymer layer. The electrode cuff was cut out of the substrate and the desired cuff and lead geometry created by thermoforming. The self-sizing cuff was made to wrap one and one half times around the nerve. The Teflon was extended at one end to form the electrode cable in the shape of a tab. The tab was 5 mm across and 1.5 cm long and provided a connection plate for the cables. Stainless steel wire (Cooner wire) with Teflon insulation was used. Conductive epoxy was used for the junction between the Teflon tab and the wire cable.

The cuffs were implanted in the upper arm, on the right median and ulnar nerves, about 8 cm above the elbow. Raccoons two through four had electrodes that had sutures at each end of the cuff to help prevent the electrode from opening and coming off of the nerve. The electrode lead was placed parallel to the nerve and distal for a few cm and then reversed and brought back and up the arm where one suture was placed around the cable to help secure it. The opposite median and ulnar nerves were exposed and isolated from surrounding tissue in all four raccoons without implanting an electrode (sham control operation).

Stimulation current was not applied to the electrodes during implantation and explantation procedures. Forty-two days after implantation, the animals were reanesthetized with an overdose of anesthetic the nerve and cuff were exposed. The cuffs were observed to determine the amount of surrounding connective tissue and the cuffs were removed from the nerves. The nerve was fixed in situ with 30 milliliters of fixative containing 5% paraformaldehyde and 5% gluteraldehyde in 0.05 M phosphate buffered isotonic saline (pH 7.4). Tissue was stored in 3% paraformaldehyde. Two to 3 mm sections of the nerve were harvested under the cuff. For sham control, a specimen was harvested from each animal at the levels corresponding to cuff implanted nerves, and all of the harvested tissues were prepared for histological examination.

Five μm semi-thin cross-sections were cut, and slides were evaluated with a microscope at magnifications of 100-400X. Cross-sections of the median and ulnar nerves at the level of the cuff were examined in regard to either signs of degeneration such as myelin fragmentation, reduction of nerve fiber density, and increased connective tissue or signs of regeneration, e.g., groups of small fibers with thinned myelin sheaths (29). Further, the cuffs were removed from the animals and observed for adherence of the sputtered multilayer metal film to the Teflon and for surface appearances with low-power light microscopy.

3.5.3 Results of Chronic Implantation Study

In an effort to limit any injury to the nerve due to manipulation during implantation both ulnar and median nerve branches were implanted in the nerve cuff. It was difficult to distinguish these three branches and postmortem evaluation revealed that the deep median branch was missing from the cuff in Raccoon #2 and it may have been absent from Raccoon #4 as no digit response to stimulation was noted.

The animals did not appear to be adversely affected by the implantation of the cuff on the median nerve. There were observed first daily and then weekly after implantation and there was no noticeable change in their use of their right upper arm. They used their right paw for grasping, eating, and hanging from the cage as before the implantation.

After six weeks of implantation the raccoons were again anesthetized and the electrodes exposed. The cuff in Raccoon # 1 had come off of the nerve and was lying 1 to 2 cm away from the nerve. It was covered with connective tissue several mm in thickness. The cables to the nerve were found to have come loose from the cuff. They may have come off during explantation procedures. Thick connective tissue was also found on the cables near the cuff. to help secure the cuff to the nerve subsequent cuffs were sutured together after they were placed on the nerve. The cuff lead connection was also improved by enlarging the contact surface area and providing strain relief. Small holes were placed along the side of the nerve and a suture was placed at both ends of the nerve to help secure it. In the remaining three raccoons the cuffs were still on the nerve at explantation. However, each of the cuffs had opened up like a 'C' being held on the nerve with the sutures. The cuffs were filled with connective tissue several millimeters thick, and the nerve were seen to lie adjacent to the cuffs.

As shown in Table 3.6, all of the raccoons responded to stimulation during initial implantation with threshold currents ranging from 0.5 to 3.5 mA. However, after six weeks of implantation, currents were not effective except at high stimulating currents with some of the electrodes (stimulation applied before viewing or explantation of the cuff). Raccoon #2 responded with high current on electrodes 1 and 4. Raccoon #3 did not respond to stimulation on any of its electrodes, and Raccoon #4 had a response with high stimulating currents on electrode 1,2 and 3.

Cross-sections of the median and ulnar nerve beneath the cuff for Raccoon #1 exhibited no sign of injury. However, the cuff had come off of the nerve in this animal. The histology of the nerves of the three remaining animals is still under investigation and can't be reported at this time.

The titanium/iridium electrode areas as well as fluorocarbon polymers appeared unchanged by the implantation. The metal remained adherent to the polymers based on visual observations.

Table 3.6. Functional responses of the volar forearm to cuff electrode stimulation.

Electrode Location	Threshold (mA)	1/2 Maximal (mA)	Maximal (mA)
Raccoon #1			
0°			
Initial	wrist flexion, 0.7	elbow, wrist, digit, 1	elbow, wrist, digit, 3
Six week	no response, 12		
90°			
Initial	wrist, digits, 1	wrist, digits, 2	elbow, wrist, digits, 3
Six week	no response, 12		
180°			
Initial	digits, 0.7	wrist, digits, 2	elbow, wrist, digits, 4
Six week	no response, 12		
270°			
initial	wrist, digits, 0.7	elbow, wrist, digits, 2	elbow, wrist, digits, 6
six weeks	no response, 12		
Raccoon #2			
0°			
Initial	ulnar digits, 3.5	wrist, ulnar digits, 4.5	wrist, ulnar digits, 6
Six week	wrist, digits, 5.2	wrist, digits, 8	elbow, wrist, ulnar digits, 15
90°			
Initial	wrist, 1	elbow, wrist, 1.8	elbow, wrist, 3
Six week	no response, 15		
180°			
Initial	ulnar digits, elbow 0.6	ulnar digits, elbow 1	upper arm, elbow, ulnar digits, 1.5
Six week	no response, 15		
270°			
Initial	elbow, 0.4	upper arm, elbow 0.8	upper arm, elbow, 1.3
Six week	pronation, 6	not reported	elbow, wrist, pronation, digits, 15
Raccoon #3			
0°			
Initial	wrist, digits, 1.4	wrist, digits, 2.5	upper arm, elbow, wrist, digits, 6
Six week	no response, 15		
90°			
Initial	wrist, digits, 1.8	wrist, digits, 2.5	upper arm, elbow, wrist, digits, 6
Six week	no response, 15		

Table 3.6 (continued)

Electrode Location	Threshold (mA)	1/2 Maximal (mA)	Maximal (mA)
180°			
Initial	elbow, 1.1	upper arm, elbow, wrist, digit, 2.5	upper arm, elbow, wrist, digit, 6
Six week	no response, 15		
270°			
Initial	elbow, 0.6	upper arm, elbow, wrist, digit, 1.5	upper arm, elbow, wrist, digit, 5
Six week	no response, 15		
Raccoon #4			
0°			
Initial	wrist, 1.5	wrist, 3	upper arm,
Six week	elbow, 8	elbow, 10	upper arm, elbow, 15
90°			
Initial	elbow, 0.5	elbow, 1.2	elbow, wrist, 2
Six week	elbow, 2.5	upper arm, elbow, wrist, digits, 5	upper arm, elbow, wrist, digits, 12
180°			
Initial	pronation, 0.5	elbow, 1	elbow,pron.,wrist, 3.5
Six week	elbow, 1	upper arm, elbow, wrist, digits, 4	upper arm, elbow, wrist, digits, 12
270°			
Initial	pronation, 0.8	wrist, 3	wrist, 6
Six week	no response, 15		

While observing the paw, a threshold response was just perceptible; the maximal response was noted when further increases in current did not appear to induce further increases in paw or forelimb movements and the half maximal response was noted and the current reported.
*most prominent response.

3.5.4 Discussion of Chronic Implantation Study

This study was conducted to quantify the short-term effects of a newly developed thin-film peripheral nerve cuff electrode on the integrity of peripheral nerves. The gross observation of the cuffs indicated that the thin-film cuff needs to be more rugged to withstand a location on the arm where there is vigorous movement. As stimulation was only applied to the electrodes during implantation, only physical and mechanical factors caused by the electrodes themselves were evaluated. The cuff came off of the nerves in the first raccoon and opened up with considerable connective tissue between the cuff and the nerve in the remaining three implants. Both of these observations indicated that a stronger cuff needs to be constructed.

Although limited nerve histology was conducted for this study. All of the animals exhibited normal paw function following cuff implantation suggesting that nerve damage would be limited.

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